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SOLITARY PULMONARY NODULES FOUND IN A COMMUNITY-WIDE CHEST ROENTGENOGRAPHIC SURVEY[1, 2, 3]

A Five-Year Follow-up Study

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Introduction

The extensive use of routine chest roentgenograms and mass chest roentgenography over the past fifteen years has stimulated an increasing interest in solitary pulmonary nodules, and numerous reports (1–11, 13–33, 35–37) have appeared concerning their diagnosis and management. Unfortunately, almost all of these reports are based on series of cases in which surgical exploration or resection had been performed so that the results apply largely to persons who sought medical attention for various reasons and who also accepted surgery when it was advised. Further restrictions on the race, sex, or age of the patients accepted for the series, or on the presence or absence of symptoms, also limit the general applicability of the conclusions which have been put forth. The variations in criteria for selection of subjects for inclusion in the various studies is reflected in the fact that the frequency of malignancy in reports reviewed varied from 5 to 74 per cent (1–5, 11).

Persons with solitary pulmonary nodules who are discovered in a mass chest roentgenographic program in which a large percentage of the total population of a community has been examined should be a fairly representative sample of such nodules as they exist in the general population. For this reason, it was decided to study the outcome in persons with solitary pulmonary nodules discovered in this manner in an effort to eliminate many of the selective factors which have entered so heavily into almost all other studies.

Method of Investigation

Selection of Patients for Follow-Up Study

The persons included in this study were selected from the participants in a community-wide mass chest roentgenographic survey which was conducted in Cuyahoga County (including the City of Cleveland) from March to September, 1949, during which almost 65 per cent of the adult population had chest films. The number of screening films taken during this survey was 673,218, of which 15,998 showed abnormalities (12). Of these abnormalities 11,572 were lesions other than cardiovascular disease. The records of the 11,572 persons with abnormal screening findings were reviewed by a clinician experienced in chest diseases. For each record that indicated even the remotest possibility of a pulmonary nodule, the screening roentgenogram was obtained and studied. Those roentgenograms that appeared to show solitary pulmonary nodules were selected for further review. At this stage the clinician made a very liberal interpretation of what constituted a solitary pulmonary nodule. On the basis of these records and roentgenograms, the clinician selected 820 persons as possibly having solitary

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pulmonary nodules. The roentgenograms of these 820 persons were subsequently reviewed by a panel of five physicians, all of whom had had experience with chest diseases. One was a radiologist, another was the clinician who reviewed the records and made the original selection of the roentgenograms.

The reviewing panel developed definite criteria for selecting the cases for further study. These criteria required that the nodule be:

1. In the lung parenchyma
2. Solitary (that is, separate and apparently different from any other lesion which might be present)
3. Roughly spherical or lobulated, and fairly well circumscribed
4. Ten millimeters or more in the longest diameter
5. Not obviously adherent to adjacent structures
6. Without definite evidence of calcification
7. Seen on a postero-anterior roentgenogram of any size taken during the 1949 survey or, if lost, any other postero-anterior film taken within six months before or after the date of the survey period

These criteria were discussed by the panel members, using illustrative roentgenograms, including some which did not fully meet the criteria, so that all members would apply the criteria as uniformly as possible.

Each panel member then selected from the 820 roentgenograms those which showed solitary pulmonary nodules which met the criteria outlined above. The voting, based on 70-mm. miniature films (except for 11 of other sizes), was made by each physician independently, none knowing beforehand how the others would vote on any film. Persons were accepted for the study if three or more of the five panel members agreed that their lesions fitted the criteria. Of the 687 accepted on this basis, 61 per cent were included by unanimous vote; 25 per cent by a four-to-one vote; and 14 per cent by a three-to-two vote.

All miniature films were later enlarged, using a special projector which compensated for the different minification factors of the various types of 70-mm. X-ray equipment used during the survey. The nodules were then measured to the nearest millimeter.

Methods of Obtaining Follow-up Data

After the study group was selected, an attempt was made to obtain detailed follow-up information for the five-year interval after the 1949 survey by means of questionnaires, personal interviews with patients either through physicians or public health nurses, clinical examinations, 14- by 17-inch roentgenograms including postero-anterior views of the chest, heavy Bucky films, lateral films, and body-section roentgenography whenever possible. When necessary, roentgenograms were borrowed from private physicians, hospitals, clinics, industrial plants, and other organizations. In 11 cases it was necessary to use 70-mm. miniature films, and in one case a 4- by 5-inch miniature film for final follow-up evaluation because it was impossible to obtain 14- by 17-inch roentgenograms.

In every case in which chest surgery had been performed or other pertinent investigations carried out, detailed information of the nature of the pathologic lesions was obtained.

Copies of death certificates were obtained for all deaths, irrespective of cause. A hospital summary of the last illness, or a report from the doctor in attendance, as well as information concerning any available laboratory, surgical, or autopsy findings, was obtained for each death.

The clinician who chose the cases for the reviewing panel evaluated and recorded the medical follow-up information of the study group. Two other persons were responsible for collection of all data and roentgenograms in the follow-up process. It is believed that this separation of duties minimized any bias which might have resulted if more follow-up emphasis had been given to the "interesting" cases.

This was the method employed to obtain information concerning the study population approximately five years after the 1949 survey film.

In the course of analyzing follow-up findings for the 687 study cases, 21 persons were excluded from the study. Two had nodules that upon measurement proved to be less than 10 mm. in diameter; the other 19 were excluded because the apparent nodules were proved to be extrapulmonary. There were such findings as artefacts, lipomas, nipple shadows, and pleural plaques. As evidence of how closely some of these shadows simulated bonafide nodules on single 70-mm. miniature films, it may be stated that, for 10 of the 19, all five readers were in agreement that the shadow represented a solitary pulmonary

TABLE 1

SELECTION OF THE STUDY GROUP

Category	Number of Persons
Satisfactory roentgenograms taken	673,218*
Possible nodules	820
Selected for study by panel	687
Excluded from study	21
Study group	666

* Includes 103 persons with unsatisfactory 70-mm. films who subsequently had satisfactory 14- by 17-inch films.

nodule. It is possible, of course, that the roentgenographic shadows of additional members of the study group were also extrapulmonary, particularly among persons for whom complete follow-up information was not available. It is believed, however, that the number of additional extrapulmonary nodules was so small that it would not materially affect any of the findings of this study.

Thus, 21 of those originally selected for study by the reviewing panel were rejected as not meeting the nodule criteria, leaving a total study group of 666 persons with solitary pulmonary nodules (table 1).

Results of Follow-up Observations

Follow-up data were collected between April 1, 1954, and June 30, 1955. On 22 of the 666 patients no information whatsoever was available since 1949 and, for 51 additional persons known to be alive at the end of the follow-up period, no roentgenograms were available since 1949 although some pertinent information about their health status was obtained. Of the remaining 593, there were 18 who did not have roentgenograms during the 1954–1955 data collection period, although other necessary information about them was obtained. Sixty-one of the 593 had died and the cause of death was verified by death certificate. The deaths were fairly evenly distributed throughout the five-year period. After subtracting both of these groups, 514 were alive concerning whom complete information was available at the end of five years.

Although the nominal length of follow-up for this study is five years, the length of the follow-up interval varies from person to person. The length of follow-up interval ranged from fifty-three to seventy-three months for these 514 persons. The average for the group was five years and three months.

TABLE 2

Completeness of Follow-up Study

	Number	Per Cent	
Total study group	666	100.0	
Alive: complete follow-up study	514	77.2	96.7
Dead	61	9.1	
Alive: incomplete follow-up study	69	10.4	
Not known whether alive or dead	22	3.3	

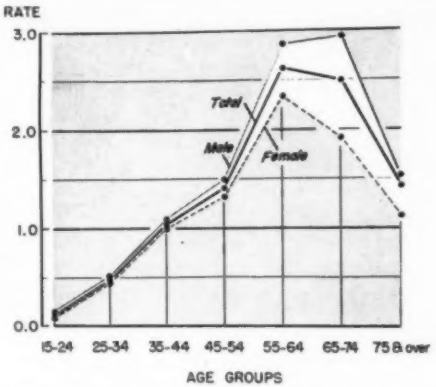

Fig. 1. Age-sex specific prevalence rates of nodules per 1,000 persons examined roentgenographically in 1949.

Those persons who had films taken during the investigating period and those who died are counted as having had complete follow-up; they comprise 86.3 per cent of the study group (table 2). Adding those persons about whom some information was available regarding health status but with no final roentgenograms, the per cent is 96.7. This leaves only 3.3 per cent for whom no information was available.

The over-all prevalence rate of nodules was 1 per 1,000 persons examined roentgenographically, and such lesions comprised 5.8 per cent of the persons recalled for suspected chest pathology (not including suspected heart disease).

Demographic Characteristics of Study Population

A comparison of age, sex, and race characteristics of the study group and the survey population from which the study group was chosen reveals some interesting relationships.

Solitary pulmonary nodules were found more frequently among older persons. This is demonstrated by the solid line in figure 1, which shows that the prevalence rate of nodules for the age group fifty-five to sixty-four was five and one-half times higher than the rate for the age group twenty-five to thirty-four.

In figure 1 it may be seen that, among the surveyed population, solitary pulmonary nodules were present more frequently among males than females in each age group, and that the contours

of the curves are similar. The rate for all males (1.12 per 1,000) was approximately one-third higher than the female rate (0.87 per 1,000). Except for those seventy-five years of age and older, the older the age group, the more marked was the difference in the sex-specific rates. This difference in the nodule prevalence rates by sex is not due to the age or the race distributions of the study group.

The prevalence of nodules also varied by race. Solitary pulmonary nodules were twice as frequent among the white as among the non-white surveyed population. The rate for the white population (1.06 per 1,000 persons surveyed) was about twice the rate for the non-white population (0.49 per 1,000). The difference in the nodule prevalence rates by race is not due to the age or the sex distributions of the study group.

Etiology

One of the objectives of this study was to ascertain the etiologic diagnoses for as many of the solitary pulmonary nodules as possible. These diagnoses were made at various times in the history of the nodules. It should be pointed out that the etiologic diagnoses of the nodules in the study group were not made by those who were responsible for this study. The function of those responsible for the study was to collect diagnoses that had been made by various individuals and agencies, with no attempt, or even opportunity, to influence the diagnosis or to cause a diagnosis to be made. For purposes of analysis the assumption has been made that these diagnoses represent the etiology of these nodules as they were first seen in 1949.

The etiology of nodules could be obtained in only 92 patients, or 13.8 per cent of the study group (table 3). One explanation for this finding might be that the causative factors for lesions not producing symptoms were less likely to be established than for lesions causing serious illness or death. This hypothesis is supported by the finding, reported in a later section, that the majority of solitary pulmonary nodules did not adversely affect the life and health of the study population.

In the five-year observation period, only 3 per cent of the study group was found to have malignant nodules. This rate is based upon 20 persons whose nodules were proved to be malignant by surgery or autopsy. An additional 7 persons had nodules which were suspected of being malignant, but adequate proof of malignancy was lacking. The diagnosis of tuberculosis was considered to be firmly established for 60 persons, or 9 per cent of the study group. While the results of histologic or bacteriologic examination were available for only some of the persons upon whom this rate is based, the evidence available for the others was so persuasive that a diagnosis of tuberculosis could be made with a high degree of confidence. An additional 64 persons also had evidence suggestive of tuberculosis, but they have not been counted as tuberculous because the evidence for such a diagnosis was not as strong. Thus, the diagnosis of definite tuberculosis was made three times as often as the diagnosis of malignancy.

While for the study group as a whole 3 per cent of the nodules were malignant, and 9 per cent were tuberculous, there were wide variations by age and by sex. There were no malignant nodules among persons younger than forty-five. In the age group forty-five to sixty-four, 4.5 per cent of the nodules were malignant; and for those age sixty-five or older, 5.8 per cent were malignant. The tuberculous nodules, however, were relatively more frequent in the younger age groups. For persons less than forty-five, 11.1 per cent of

TABLE 3

PROPORTION OF STUDY GROUP WITH KNOWN ETIOLOGY, BY SEX

Sex	Total Study Group		Known Etiology							
			Total		Malignancies		Tuberculosis		Other Disease	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	666	100.0	92	13.8	20	3.0	60	9.0	12	1.8
Male	380	100.0	50	13.2	16	4.2	26	6.8	8	2.1
Female	286	100.0	42	14.7	4	1.4	34	11.9	4	1.4

TABLE 4

PERCENTAGE DISTRIBUTION BY ETIOLOGY OF NODULE-SIZE GROUPS

1949 Nodule Diameter	Study Group		Unknown or Suspected Etiology		Known Etiology							
					Total		Malignancy		Tuberculosis		Other Diseases	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	661*	100.0	570*	86.3	91*	13.8	19*	2.9	60	9.1	12	1.8
Less than 25 mm.	462	100.0	428	92.6	34	7.4	2	0.4	30	6.5	2	0.4
25–39 mm.	156	100.0	119	76.3	37	23.7	7	4.5	26	16.7	4	2.5
40 mm. and more	43	100.0	23	53.5	20	46.5	10	23.2	4	9.3	6	14.0

* Persons with unknown nodule size in 1949 are excluded.

the nodules were tuberculous. In the age group forty-five to sixty-four, 8.0 per cent were tuberculous; and for those sixty-five or older, 7.0 per cent were tuberculous. Malignant nodules were found less frequently in each age group than were tuberculous nodules.

As is shown in table 3, the per cent of malignant nodules was three times as high among men as among women, whereas the per cent of tuberculous nodules was only half as great among men as among women.

Size of the Nodule

The size of solitary pulmonary nodules, as determined from the roentgenograms taken in 1949, ranged from 10 to 115 mm. Of the 661 persons for whom the nodule size of 1949 was available, 199 (30.1 per cent) had nodules which measured 25 mm. or more and 43 (6.5 per cent) had nodules with measurements of 40 mm. or more (table 4).

The determination of the etiology of the nodule was directly related to nodule size. Etiology was known for only 7.4 per cent of those less than 25 mm. in diameter, whereas etiology was known for 46.5 per cent of those more than 40 mm. in diameter. This is a reasonable finding, since it is likely that the larger nodules will cause more concern in both physician and patient, with resultant greater efforts at diagnosis. In addition, if it is presumed that the malignant nodules will generally pursue an inexorable course toward a symptomatic or fatal termination, and if the larger nodules are also more likely to be malignant as has been reported by others (15, 16), this combination of characteristics would also explain the higher proportion of large nodules with known etiology. The findings of this study do not support or refute the hypothesis that malignant nodules invariably become symptomatic or fatal, but they do support the second hypothesis. The average size of the malignant nodules (52 mm.) was twice that of the tuberculous nodules (25 mm.) and two and one-half times that of all other nodules (21 mm.).

As is shown in table 4, 7.4 per cent of the 462 nodules less than 25 mm. in diameter had known causes. Only 2 (0.4 per cent) were malignant, and 30 (6.5 per cent) were tuberculous. Among the 43 largest nodules, 40 mm. and more in diameter, nearly half had known etiology, 10 (23.2 per cent) being malignant and 4 (9.3 per cent) being tuberculous. Thus, there is a marked increase in the proportion of nodules found to be malignant with an increasing size of nodule. The increase in the diagnosis of tuberculosis with an increasing size of nodule is much less marked.

Changes in Nodule Size

Of the 514 persons who were alive at the close of the study and had had complete five-year follow-up study, 469 (91.2 per cent) still had nodules. Disappearance or change in character of the other 45 nodules was due to surgery in about one-third, resolution or residual fibrosis in one-half, and there were various other reasons for the remainder.

Approximately half of those persons still presenting solitary nodules after the five-year interval showed no change in nodule size; 30 per cent demonstrated a decrease in nodule size; and, in 20 per cent, there was an increase in nodule size. Information with respect to nodule changes was not available at the close of follow-up study for the 61 deaths, for the 69 persons with less than complete follow-up information, or for the 22 persons not known to be dead or alive.

Almost two-thirds of the tuberculous nodules were present at the end of the five-year period, and one-third of them had disappeared or lost nodular character due to surgery, resolution, spread, or residual fibrosis. Of the 37 tuberculous nodules present at the close of follow-up observation, 40 per cent showed some change in size.

No information about change in nodule size was available for malignant nodules because all persons with proved malignant nodules either died or had the nodules resected before the end of the five-year follow-up period. Although interim roentgenograms (after 1949 but before the data collection phase of this study) were available for some patients, it was not considered practicable to use these data in the analysis of nodule size changes.

Location of Nodule

Solitary pulmonary nodules were found more frequently in certain lung areas than in others. They were found more frequently in the right than in the left lung, with 61 per cent in the right lung and 39 per cent in the left lung. Eight per cent of the nodules in the right lung were tuberculomas, whereas in the left lung 11 per cent were so diagnosed. Carcinoma was diagnosed somewhat more frequently among the nodules in the right lung (3 per cent) than in the left lung (2 per cent).

More nodules were found in the lateral than in the medial portions of the lung, but the etiology of the medial nodules was more frequently identified. The lateral portions of the lung contained 68 per cent of the nodules and the medial 32 per cent. Among medial nodules, 13 per cent were tuberculosis and 4 per cent cancer, whereas among lateral nodules only 7 per cent were tuberculosis and 2 per cent cancer. Although some of these differences look impressive, the only one that could not easily be explained as a chance occurrence was the observation that medial nodules were more frequently tuberculous than were lateral nodules. It is apparent that the location of the nodule gave no reliable clues to its etiology.

Border Characteristics

One of the criteria for selection was that the nodule must have appeared, on the selection film, to be "fairly well circumscribed." The 666 persons who constitute the basic study population were subsequently divided into two categories according to whether the nodule appeared, in 1949, to be well circumscribed or not so well circumscribed. The latter category is called "poorly circumscribed" for convenience in the discussion which follows. It is obvious that the decision to include a case in one or the other of these groups was quite subjective. All study cases, however, were well enough circumscribed to satisfy the selection criteria.

Of the total of 666 persons, 68 per cent had well-circumscribed lesions and 32 per cent had poorly circumscribed lesions in 1949 (table 5).

The etiology of the nodule was determined in 17.6 per cent of those whose nodules were poorly circumscribed and in 11.9 per cent of those with well-circumscribed nodules. This indicates either that poorly circumscribed nodules are thought to be more dangerous than well-circumscribed nodules and, therefore, are most likely to be in-

TABLE 5

RELATIONSHIP OF BORDER CHARACTERISTICS OF NODULES TO DEATH AND SYMPTOMS

Border of Nodule 1949	Study Group		Alive										Not Known Dead or Alive		Dead					
			Total		No Symptoms		Symptomatic		Apparently Well		Health Status Unknown				Total		Nodule Cause of Death		Nodule Not Cause of Death	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	666	100.0	583	87.5	536	80.5	23	3.5	7	1.0	17	2.6	22	3.3	61	9.2	17	2.6	44	6.6
Well-circumscribed	454	100.0	403	88.8	371	81.7	13	2.9	5	1.1	14	3.1	15	3.3	36	7.9	9	2.0	27	5.9
Poorly circumscribed	212	100.0	180	84.9	165	77.8	10	4.7	2	1.0	3	1.4	7	3.3	25	11.8	8	3.8	17	8.0

vestigated intensively, or that the etiology of poorly circumscribed nodules is more easily established, possibly because they more often led to symptoms or death. The poorly circumscribed nodules were somewhat more frequently diagnosed tuberculosis or cancer than were the well-circumscribed nodules, but the differences were not statistically significant.

Nodule Shape

Approximately 60 per cent of nodules were round and 40 per cent were oval. Although the determination of shape is highly subjective, there appears to be some difference related to the shape of the nodules which were diagnosed as tuberculous. A slightly higher proportion of oval than round nodules was diagnosed as tuberculous, but this difference was not statistically significant.

Rigler (18) attributes diagnostic significance to nodules with umbilicated borders. The findings reported above neither dispute nor confirm his findings. His study depended upon body-section roentgenography to determine small border details. In the study reported here body-section roentgenography was done on only one-third of the study population, and the detail necessary to test Rigler's hypothesis was not recorded.

Calcification

One of the case-selection criteria for this study was that the nodule must not have "definite evidence of calcification." This criterion was agreed upon because it was not possible in this study to include all persons, who, in 1949, had calcified solitary pulmonary nodules. Some persons whose nodules showed definite evidence of calcification had been classified as "essentially negative" at the time of the Cuyahoga County community-wide chest roentgenographic survey. It was not feasible, for the present study, to attempt to recover all or a representative sample of persons with calcified lesions from among those read as "essentially negative." It was, therefore, decided that the review panel would exclude from this study any person whose nodule appeared obviously calcified on the 1949 miniature film. This policy did not prevent the inclusion of nodules which were possibly calcified. It is not surprising, therefore, that the number of nodules subsequently shown to be calcified by techniques more refined than the miniature film was rather high. Subsequent studies such as body-section roentgenograms and additional postero-anterior roentgenograms demonstrated that lesions in 207 persons, 31 per cent of those chosen for study, were definitely or probably calcified in 1949. It is worthy of note that 83.6 per cent of these 207 lesions originally were considered by four or more panel members not to be obviously calcified.

Eight per cent of noncalcified nodules caused symptoms or death. Only about one per cent of the calcified nodules caused symptoms and none of them caused death.

Also interesting, because it supports the generally prevailing opinion that a calcified solitary pulmonary nodule is not likely to be malignant (9–11, 15, 16, 20, 24–26, 35, 37), is the observation that none of the proved malignant nodules had demonstrable calcification.

Malignancy and Selection of Study Groups

The finding of only 20 persons (3 per cent) of the total study group with diagnoses of malignant nodular lesions is in contrast to other reports which have shown that larger percentages of solitary pulmonary nodules are malignant (2, 5, 10, 29–33). Even by excluding the calcified and probably calcified nodules, the malignancy rate increases to only 4.4 per cent. Table 6 was prepared by excluding specified categories from the study population. It shows the risk of malignancy in the remaining population in each instance. It will be noted that the range was from 3 per cent in the total study population to 23.3 per cent in persons with nodules of more than 39 mm. When the exclusions shown in table 6 are combined so that all that remain are males more than forty-four years old with complete follow-up data, who have uncalcified nodules of more than 39 mm. in greatest diameter, only 23 persons remain. Eight of these have malignant nodules. This yields a rate of 35 per cent and shows how selectivity of the population base can produce high rates of malignancy.

The elimination of certain population groups as outlined above is the implicit process employed in the study design of some previously published reports as discussed by Paulson (16). One exception of which the present writers are aware is the paper by Comstock and co-workers (11) which is based on a group that approximates a general population in composition, as does this study. Most others start with a pre-selected population.

TABLE 6

MALIGNANCY IN SELECTED STUDY SUBGROUPS

Independent Exclusions	Number	Malignancies	
		Number	Per Cent
Total	666	20	3.0
Number with follow-up information	593*	20	3.4
Excluding males less than 45	535	20	3.7
Excluding females	380	16	4.2
Excluding well-circumscribed nodules	212	9	4.2
Excluding calcifications	459	20	4.4
Excluding nodules less than 20 mm. in diameter	337	19	5.6
Excluding nodules less than 25 mm. in diameter	199	17	8.5
Excluding nodules less than 30 mm. in diameter	117	14	12.0
Excluding nodules less than 35 mm. in diameter	64	11	17.2
Excluding nodules less than 40 mm. in diameter	43	10	23.2
Combined exclusions (*see* text)	23	8	35.0

* Excluded from this total are 51 persons for for whom there is no follow-up information except that they are alive, and 22 persons whose status was completely unknown.

Such studies are useful and can test hypotheses that are beyond the scope of this study. On the other hand, they are inadequate to indicate the proportion of all solitary pulmonary nodules which will cause illness or death. They are also inadequate to indicate the demographic and physical characteristics which may be helpful in identifying those persons in whom nodules may be dangerous.

Health Status and Mortality with Respect to Nodules

There were 536 persons (80.5 per cent) of the entire study group who lived five years after their nodules were discovered and who never had any illness attributable to the nodule (table 5 and figure 2). This includes 60 persons for whom follow-up information was less complete than for most of the study population. However, these 60 persons were known to have had no symptomatic illness due to nodules in the five-year period.

Twenty-three persons (3.5 per cent) had had symptoms attributed to the nodules but were alive at the time of their five-year follow-up study. Seventeen of them had tuberculous nodules. Two nodules were malignant, and the remaining four were diagnosed as possibly cyst, pneumonia, benign tumor, and suspected tuberculosis. At the conclusion of the follow-up period 18 of the 23 persons had recovered. The remaining 5 persons were ill due to pulmonary tuberculosis caused by spreads presumably originating from the nodules.

Deaths traceable to nodules as the underlying or contributing causes accounted for 17, or only 2.6 per cent, of the study population. A further discussion of the deaths will be found later in this paper.

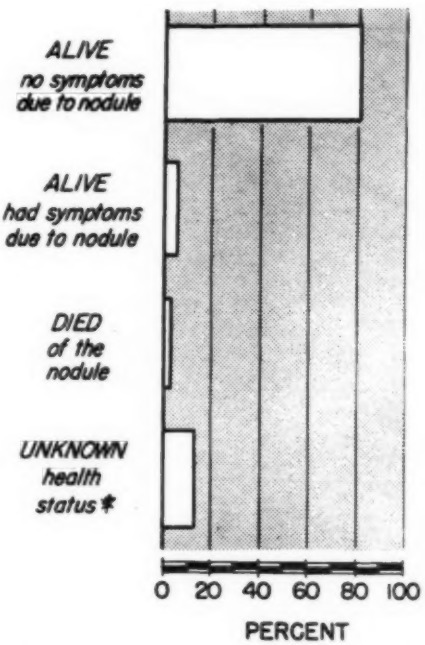

FIG. 2. Health summary of study population at end of five years.

* Comprised of 7 persons who may or may not have had symptoms during the five-year interval but who were apparently well at the end of the follow-up period; 17 persons who were known to be alive at the end of the follow-up period, but whose health status was unknown for the entire period; 22 persons not traced; and 44 persons who died of causes not related to the nodule.

There were ninety persons (13.5 per cent) in whom health status in regard to the nodule was undetermined. This includes 44 persons who died of causes not related to nodules and whose health status with respect to nodules was unknown, 22 persons unknown to be either alive or dead at the time of follow-up study, and 24 persons who were alive at the close of follow-up but whose health status was unknown. Even if it is assumed that all of these 90 persons had had illness or death due to nodules, then only 19.7 per cent of the total study population could have suffered illness or death resulting from nodules in the five-year period. On the other hand, if it is assumed that not one of the 90 persons with undetermined health status had illness or died because of nodules, then in only 6.2 per cent of this study group did nodules have an adverse effect. These two assumptions, however, are extreme. Considering only those 576 persons whose health status was established, 7.1 per cent had illness or died because of nodules. Thus, while the range of possible values of the percentage of persons who died or were ill because of nodules extends from 6.2 to 19.7 per cent, a more reasonable estimate is 7.1 per cent.

The mortality rate among those persons in whom nodules were the underlying or contributing causes of death (table 5) was higher for those with poorly circumscribed nodules than for those with well-circumscribed nodules. The percentages are 3.8 and 2.0, respectively. This is a ratio of almost twice as many deaths among persons with poorly circumscribed nodules as among those with well-circumscribed nodules. For those persons who died of causes not related to the nodule, the ratio of deaths among the poorly circumscribed group as compared to the well-circumscribed group was only one-third higher.

As stated earlier in this article, there were sixty-one persons in the study group who died from all causes during the five-year observation period. This is 9.5 per cent of the study group for whom information was available at the close of follow-up study. Almost half of these deaths were caused by disease of the cardiovascular system, and approximately one-fourth were caused by malignant disease of the lung. No one in this study died of tuberculosis. However, approximately one-fifth of the persons who died were known to have had tuberculosis. Seventeen of the 61 deaths were caused by malignant pulmonary nodules, a mortality of 2.6 per cent for the cause of death in the total study group. Nodular lesions caused no deaths except those due to malignancy. Sixteen of the malignant nodules were primary bronchogenic carcinomas. In one of these 16, the patient had a progressing lung cancer but actually died of peritonitis. For the purpose of this study, lung cancer is considered the cause of death. One patient had a nodular lesion due to Hodgkin's granuloma and eventually died of this disease.

It is possible that some or all of the 22 persons who were not located during the follow-up period had also died. If all of them died, the total number of deaths would be about one-third higher and the increase for certain causes might be still greater. (For example, if they had all died of pulmonary malignancy, the number of deaths from the cause would be more than doubled.) Because extensive effort was spent on follow-up data in this study some information is available about these 22 persons. That information indicates that it is unlikely that more than one or 2 of them died. With these considerations in mind, analysis of mortality has been based only on known deaths. No estimates have been made of mortality among persons not located during the follow-up period.

The mortality percentages mentioned above are for the five-year period of the study. Using the method suggested by Sartwell (34), the expected number of deaths was computed for the study group.

The expected number of deaths in the study group is 52. The observed number was 61. The difference was not statistically significant. The expected number of deaths from pulmonary malignancy is less than one, while the actual number of such deaths in the study group was 17. This difference is statistically significant. The expected number of deaths from causes other than pulmonary malignancy is approximately 52; the actual number was 44 (figure 3). The difference is not statistically significant.

Reasonable inferences from these data are: (*1*) the observed number of deaths attributed to pulmonary malignancy will be substantially higher in a group composed entirely of persons with solitary pulmonary nodules than in a population in which only one person in a thousand has such nodules identified; and (*2*) the presence of

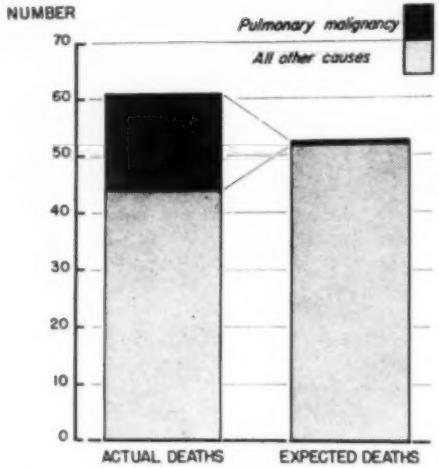

FIG. 3. Comparison of actual and expected number of deaths in the study population.

solitary pulmonary nodules does not substantially change the number of deaths from causes other than pulmonary malignancy.

DISCUSSION

The most important finding in this study is that the percentage of solitary pulmonary nodules which were proved to be malignant at the end of a five-year period of observation was much smaller than the percentage found in other published studies. One exception is the report by Comstock and associates (11), whose paper shows a very remarkable agreement in most respects with this report. It is interesting to note that the methodology of both studies was quite similar, although both were done entirely independently. For the most part, the other literature reviewed indicated that there had been a very high degree of selection of patients, and this undoubtedly is the reason for the very large discrepancy between the number of malignant nodules reported in this paper as contrasted with most other published reports. It is possible that some of the disagreement with most other studies may be related to the fact that some of the currently undiagnosed nodules may eventually be diagnosed as malignant. However, since all known malignant neoplasms manifested themselves before the fourth year, and all but three in the first two years, it is believed that the number of malignancies in this study group will not increase greatly with the passage of time.

It is not the purpose of this paper to determine which patients with solitary pulmonary nodules should have surgery. Rather it is hoped that the material presented will furnish the physician with some criteria indicating in general the groups most likely to have malignant pulmonary nodules. The problem of surgery then becomes a highly individualized decision in each case, based (among other factors) on the general condition of the patient and his age. The risk of malignancy among an unselected group of solitary pulmonary nodules does not seem clear enough to warrant routine excision.

SUMMARY

In 1949, a mass chest roentgenographic survey was conducted in Cleveland and Cuyahoga County, Ohio, during which 673,218 screening films were taken, representing a coverage of almost 65 per cent of the adult population. In 1954, the records and films of persons with abnormal findings were reviewed. From this group, 666 persons whose lesions met certain predetermined criteria were classified as having solitary pulmonary nodules and were selected for a five-year follow-up study. All but 22 persons (3.3 per cent of the total) were traced.

The over-all prevalence of solitary pulmonary nodules was one per 1,000 persons examined roentgenographically. Nodules were observed more frequently among older persons, males, and whites.

The etiology had been established for only 13.8 per cent of the study group. Only 3 per cent of the nodules were proved to be malignant, and 9 per cent were diagnosed as tuberculous. The larger nodules were much more frequently malignant than were the smaller nodules. No nodules with demonstrable calcification were found to be malignant.

Of the entire study group, 80.5 per cent of the subjects were alive and had had no symptoms attributable to the nodule during a five-year period following the original survey. Only 2.6 per cent of the study group had died of causes related to the nodule, all of the deaths being attributed to pulmonary malignancy. In all, 61 deaths were observed, whereas 52 would have been expected on the basis of the mortality experience of Cuyahoga County. The excess mortality was entirely attributable to pulmonary malignancy.

Evidence is presented to show that the proportion of malignancies among a group of persons with solitary pulmonary nodules will vary markedly according to the factors used in selecting the study group. While the proportion of malignancies in the total study group was only 3 per cent, it was 35 per cent among males of forty-five and older whose nodules showed no evidence of calcification, were poorly circumscribed, and measured 40 mm. or more in diameter. It is concluded that the decision for surgical removal of solitary pulmonary nodules must be a highly individualized decision in each case.

Sumario

Nódulos Pulmonares Solitarios Descubiertos en una Encuesta Torácica Colectiva: Estudio Quinquenal

En 1949, se llevó a cabo una encuesta colectiva con radiografías del tórax en la ciudad de Cléveland y el Condado de Cuyahoga, en Ohio, durante la cual se tomaron 673,218 radiografías de despistaje, representando una proporción de casi 65 por ciento de la población adulta. En 1954, se repasaron los protocolos y las radiografías de las personas que mostraban hallazgos anormales. De este grupo, se clasificó a 666 personas cuyas lesiones se conformaban a ciertas pautas predeterminadas como portadoras de nódulos pulmonares solitarios, escogiendóselas para un estudio de cinco años de observación. Aparte de 22 (3.3 por ciento del total), se localizó a todas ellas.

La frecuencia global de nódulos pulmonares solitarios fué de uno por 1,000 personas examinadas radiográficamente. Se observaron nódulos más frecuentemente entre las personas de mayor edad, los varones y los sujetos de raza blanca.

No se había establecido la etiología más que para 13.4 por ciento del grupo estudiado. Sólo 3 por ciento de los nódulos resultaron ser malignos, y 9 por ciento fueron diagnosticados como tuberculosos. Los nódulos mayores fueron mucho más frecuentemente cancerosos que los más pequeños. Ningún nódulo que mostraba calcificación resultó ser maligno.

De todo el grupo de estudio, 80.5 por ciento se encontraban vivos y sin síntomas imputables a los nódulos durante un período de cinco años consecutivo a la encuesta primitiva. Solamente 2.6 por ciento del grupo estudiado habían fallecido de causas relacionadas con los nódulos, atribuyéndose todas las muertes a cáncer pulmonar. En conjunto, se observaron 61 muertes, en tanto que cabía esperar 52 a base de la mortalidad experimentada en el Condado de Cuyahoga. El exceso de mortalidad fué totalmente imputable a cáncer pulmonar.

Preséntanse pruebas demostrativas de que la proporción de cáncer en un grupo de personas que tengan nódulos solitarios del pulmón variará notablemente conforme a los factores usados para escoger el grupo de estudio. Aunque la proporción de cáncer en el grupo total de estudio no pasó de 3 por ciento, llegó a 35 por ciento entre los varones de cuarenta y cinco años o más de edad, cuyos nódulos no mostraban signos de calcificación, estaban mál circunscritos y median 40 mm. o más de diámetro. Dedúcese que debe individualizarse sobremanera en cada caso la decisión relativa a la extirpación cruenta de los nódulos solitarios del pulmón.

Resume

Nodules pulmonaires solitaires décelés au cours d'une enquête municipale comportant la radiographie pulmonaire en série: Une période d'observation de cinq années

En 1949, une enquête comportant la radiographie pulmonaire de masse a été effectuée à Cleveland et Cuyahoga County, Ohio, aux U.S.A.; au cours de celle-ci 673.218 films de dépistage ont été pris, englobant 65% de la population adulte. En 1954 les dossiers et les clichés concernant les sujets présentant des signes anormaux ont été passés en revue. Parmi ceux-ci, 666 sujets dont les lésions correspondaient à certains critères prédéterminés ont été classifiés comme porteurs de nodules pulmonaires solitaires et choisis pour être inclus dans une étude de cinq années. Hormis 22 personnes (3,3% de la totalité) tous ces sujets ont été retrouvés.

La prédominance, pour l'ensemble des nodules pulmonaires solitaires chez les sujets radiographiés, est de 1 pour mille. Les nodules ont été observés plus souvent parmi les sujets plus âgés, les hommes, les Blancs.

L'étiologie n'a été établie que chez 13,8% du groupe étudié. La malignité n'a été démontrée que dans 3% des nodules et, dans 9% d'entre eux, l'étiologie tuberculeuse a été diagnostiquée. La malignité a été plus souvent décelée dans les grands nodules que dans les petits. Aucun nodule à calcification apparente n'a été trouvé de nature maligne. Pour l'ensemble du groupe étudié, 80,5% des sujets étaient vivants de n'avaient présenté aucun symptôme imputable au nodule durant la période de cinq années succédant à l'enquête initiale. Le pourcentage de décès dont la cause a été rapportée au nodule n'a été que de 2,6% pour le groupe étudié, tous les décès étant attribués à une néoplasie pulmonaire. En tout, 61 décès ont été enregistrés, alors que sur la base des taux de mortalité de Cuyahoga County 52

étaient prévus. La dégénerescence maligne était entièrement responsable de l'excédent de mortalité.

Les auteurs présentent des données montrant que la proportion des cancers parmi un groupe d'individus porteurs de nodule solitaire varie considérablement suivant les facteurs utilisés dans la sélection du groupe étudié. Tandis que la proportion des cancers pour la totalité du groupe à l'étude n'était que de 3%, elle était de 35% chez les hommes âgés de quarante-cinq ans et plus dont les nodules mal délimités avaient un diamètre de 40 mm ou plus et ne présentaient aucun signe de calcification. Les auteurs concluent que la résection chirurgicale des nodules pulmonaires solitaires exige une décision hautement individualisée dans chaque cas.

Acknowledgments

The writers are indebted to Dr. Armand Brodeur, Dr. George Badger, Dr. Jean Pilcher, Mrs. Clarice P. Buss, Mr. Mozart Spector, Miss Violet Freiler, and Miss Mary Price for assistance in preparation of material for this paper. The writers also wish to thank all of the physicians, public health departments, and hospitals who cooperated in the follow-up study of this group of patients. The number of cases for which complete information was made available would not have been nearly as large without the cooperation of many private physicians, health officers, nurses, and other health workers in Ohio and the other states to which the subjects of the study had moved during the five-year period.

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THE PATHOLOGY OF 1957 (ASIAN) INFLUENZA[1, 2]

DONALD D. MARK

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Introduction

The experience gained from the 1957 influenza outbreak has afforded an opportunity to re-examine an old question of whether there is as yet exact knowledge concerning the specific lesions of influenza virus in man. The gravity of the 1957 outbreak of Asian variant influenza type *A* may be judged by the extraordinarily high attack rate, reaching 70 per cent in some localities, and the excess in expected mortality rates attributable to influenza and pneumonia (1) (figure 1). In the State of Maryland, the epidemic struck in September and reached a peak in November, lasting altogether about seven weeks. Of 49,000 cases recorded, 130 were confirmed, the majority by serologic methods. Three hundred and sixty-eight deaths were claimed by the epidemic (2).

Since the great pandemic of 1918, the pathology of influenza has been exhaustively studied. In a catalogue of the numerous reports and monographs of the period are represented the foremost names in the history of American pathology (3–7). Since the causative agent was not established at that time, it is not unnatural for differences to have arisen in the interpretation of pathologic alterations in the lungs in fatal cases. Pfeiffer's view of the primacy of the so-called influenza bacillus, first advanced after the 1889 influenza epidemic (8), was opened to challenge during the World War I influenza outburst by the results of numerous bacteriologic studies which were obviously at variance with it.

The isolation in 1933 by Smith, Andrewes, and Laidlaw (9) of a virus of human influenza cleared away all doubt as to the essential character of the disease and provided the necessary tool for assessing the train of tissue changes in infected persons. In 1937, Straub described the course of influenza viral infection in the tracheobronchial tree and lung in mice (10). The changes consisted of early engorgement, necrosis, and squamous regeneration of the respiratory epithelium, blockage of bronchioles by exudate, and intensive destruction of bronchioles from which the lesion radiated out, accompanied by interstitial and alveolar hemorrhage and edema.

In man it has not yet been demonstrated that influenza virus unaccompanied by bacterial superinfection will produce a necrotic epithelial reaction in the tracheobronchial tree (11). However, the pulmonary findings in the 1918 pandemic as well as in more recent influenza outbreaks, complicated as they may be by bacterial overgrowth, command attention through certain resemblance to the lesions of influenza described in the ferret (12) and other species. The observations drawn from the 1957 influenza outbreak support and strengthen the view that there are gross and microscopic tissue changes which indicate an influenzal infection even when extensive alterations ascribable to bacteria also are present.

In this report, pertinent data accumulated by the study of 17 deaths which occurred during the 1957 epidemic period are presented. The virus was isolated in 6 cases from the lung, and the existence of an influenzal viral infection was proved in 4 cases by hemagglutination antibody detection. In the remaining 8 deaths, laboratory evidence of viral infection was either not proved or not attempted, but the pathologic findings are typical enough to warrant inclusion in the study.

Observations

Illustrative Case Summaries

Case 1 (JHH A-27264). M. S., a 34-year-old female, was admitted to the hospital with a history of a chest cold and headache beginning on October 5. This progressed to chills and fever on October 8. On admission to the hospital on October 9, her temperature was 104°F., pulse was 136 per minute, respirations were 50 per minute, blood pressure was 130 systolic, 60 diastolic in mm. of mercury. The patient's condition deteriorated within twenty-four hours with exaggeration of existing acute distress and increasing cyanosis and semi-stupor. Generalized crackling rhonchi were heard with breath sounds often palpable through the

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[2] Presented at the 44th Annual Meeting of the Southern Tuberculosis Conference, Southern Trudeau Society, Richmond, Virginia, October 4, 1958.

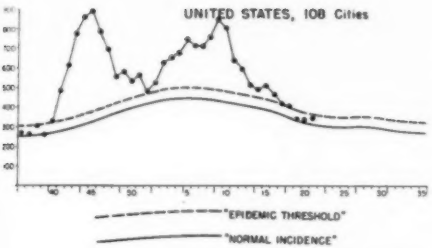

FIG. 1. The incidence of deaths attributable to influenza during the fall of 1957 and winter of 1958.

chest wall. On October 10, there was sudden cardiac arrest followed by open heart resuscitation. On October 11, cardiac arrest occurred again, with no response to massage. One sputum culture showed type XVIII pneumococci, but repeated cultures were negative for respiratory pathogens. The highest leukocyte count was 12,100 per cu. mm. A total of 44,300,000 units of penicillin and 2.5 gm. of streptomycin was administered.

Autopsy was performed five hours post mortem (Dr. Frost). The pleural cavities showed no excess of fluid; there were old fibrous adhesions over the right lung and fibrinous pleurisy in the left upper lobe. The lungs weighed 1,450 gm. No gross alterations were observed in the trachea, but the bronchi contained hemorrhagic mucoid material. Microscopically, a necrotizing tracheobronchitis and bronchiolitis with foci of squamous metaplasia were found. The lungs were generally red-brown, firm, and succulent, with large confluent patches of red and mottled yellow consolidations in all lobes. Microscopically, in addition to diffuse serofibrinous pneumonia showing moderate mononuclear infiltration, hyaline membranes, and epithelization of alveoli, there was focal early suppurative pneumonia. Influenza virus type A (Asian strain) was cultured from the lung.

Case 2 (BCH A-16704). L. R. J., a 17-year-old male, had a history of fever followed by sore throat from October 5 to 7. Chest pain and shortness of breath commenced on October 9. He was admitted to the hospital on October 10 in severe acute respiratory distress with a temperature of 103° F.; his pulse was 132 per minute, respirations were 60 per minute, and blood pressure was 110 systolic, 90 diastolic in mm. of mercury. Bilateral rales, loud rhonchi, and friction rubs were present over the lung fields. Therapy consisted of penicillin, tetracycline, and streptomycin. Pericardial and left pleural taps on October 11 yielded straw-colored fluid containing coccal forms. This fluid yielded hemolytic *Staphylococcus aureus* on culture. On that day, a sudden afternoon temperature rise to 105° F. occurred which was followed by apnea and cardiac arrest, but the patient responded to thoracotomy and cardiac massage. Large effusions were drained from the left chest and pericardium. Another episode of cardiac arrest occurred during the evening of October 11 with, however, no response to cardiac massage.

Autopsy was performed twelve hours after death (Dr. Stazio). The right pleural cavity contained 1,000 ml. of straw-colored fluid. The pericardial cavity and left pleural cavity were obliterated by matted fibrinous adhesions. The lungs weighed 2,100 gm. and were coated with fibrin. The cut surface was dense, red, firm, and congested with innumerable small abscesses scattered throughout the lobes. The trachea and bronchi were swollen, edematous, and roughened by irregular patches of fibrinopurulent exudate. The smaller bronchial subdivisions lay virtually occluded by exudate. Microscopically, a necrotizing and membranous inflammation disrupted and obstructed the entire tracheobronchial tree. Focal remnants of persisting respiratory mucosa were transformed or regenerated into transitional and squamous epithelium. Massive destruction of bronchiolar walls was marked in numerous areas, generally associated with suppuration of surrounding pulmonary lobules. Coalescence of these regions and destruction of vascular channels had led to abscess cavities and pulmonary hemorrhage which involved most of the lung substance. Culture of the lung post mortem for influenza virus was negative. A heavy growth of *Staphylococcus aureus* was reported.

Case 3 (BCH A-16841). M. B., a 51-year-old female, had a four-month history of exertional dyspnea and pedal edema. She was admitted to the hospital on December 15 in auricular fibrillation, and her condition responded to digitalis and diuretics. She became abruptly febrile on December 22 and 25, and penicillin, chloramphenicol, and streptomycin therapy was begun. Despite treatment, progressive tachypnea and cyanosis ensued which were followed by an episode of sudden shock and anuria. The leukocyte count was 22,000 per cu. mm. She gradually became semi-stuporous and developed coarse inspiratory and expiratory rales. On December 29, some rise in blood pressure and improvement in sensorium were noted with the onset of diuresis, but tachypnea and tachycardia persisted despite adequate digitalization. The patient died on January 1.

Autopsy was performed thirteen hours post mortem (Dr. Kramer). There were 200 ml. of serosanguineous fluid in the right pleural cavity. The lungs weighed 1,300 gm. and appeared uniformly edematous and without consolidations. The tracheobronchial tree was congested and roughened by flakes of firmly attached fibrinopurulent exudate. The heart was enlarged, weighed 440 gm., and showed narrowed coronary vessels and diffuse myocardial fibrosis. Visceral passive congestion was noted. Acute renal tubular necroses, acute peptic erosions of the stomach, and focal acute necrosis of the anterior hypophysis were found, all the probable result of ischemia (shock).

TABLE 1

PATHOLOGY ASSOCIATED WITH ASIAN INFLUENZA

Patient Age, Sex	Date	Tracheobronchitis Squamous Metaplasia	Bronchiolitis	Serofibrinous Pneumonia	Suppurative Pneumonia	Bacteriology	Influenza Virus Studies	
							Isolation	Serologic
M. S. 34, F.	10/19	+	+	+ Hyalin membranes, alveolar epithelization	0	Pneumococcus, type XIII: sputum	+	----
E. H. 61, M.	11/19	+	+	+ Hyalin membranes, alveolar epithelization	0	----	+	----
W. B. 72, M.	11/19	+	0	0	0	Staphylococcus: sputum	----	----
G. B. 40, M.	10/10	+	+	0	+	Staphylococcus: nose, lung	----	----
N. L. 21, M.	10/17	+	+	0	+	Staphylococcus: lung	+	----
M. B. 51, F.	12/15	+	0	0	0	----	----	----
H. O. 45, M.	10/19	+	+	0	+	Staphylococcus: sputum	0	----
M. D. 32, F.	11/2	+	+	0	+	Staphylococcus: lung	0	----
L. J. 17, M.	10/10	+	+	0	+	Staphylococcus: pericardium	0	----
* E. N. 15, F.	10/15	+	+	+	0	Staphylococcus: lung	+	----
* L. L. 34, F.	10/21	+	+	+ Hyalin membranes	+	Staphylococcus: lung	+	----
* A. S. 50, F.	10/29	+	+	+ Hyalin membranes, alveolar epithelization	0	Staphylococcus: lung	0	1:40
* N. H. 13, F.	11/13	+	+	+ Hyalin membranes	0	Staphylococcus: lung	----	----
* R. K. 15, M.	11/2	+	+	+	0	Sterile†	----	1:80
* C. G. 34, F.	10/13	+	+	+ Hyalin membranes	0	Pneumococcus, type III: lung	0	1:40
* R. M. 17, F.	11/17	+	+	+ Hyalin membranes, alveolar epithelization	0	----	----	----
* A. T. 26, F.	11/16	+	+	0	+	Staphylococcus: sputum	+	1:40

---- Refer to studies not done.

* Case previously presented (*see* text footnote[3]).

† Received no antimicrobial therapy.

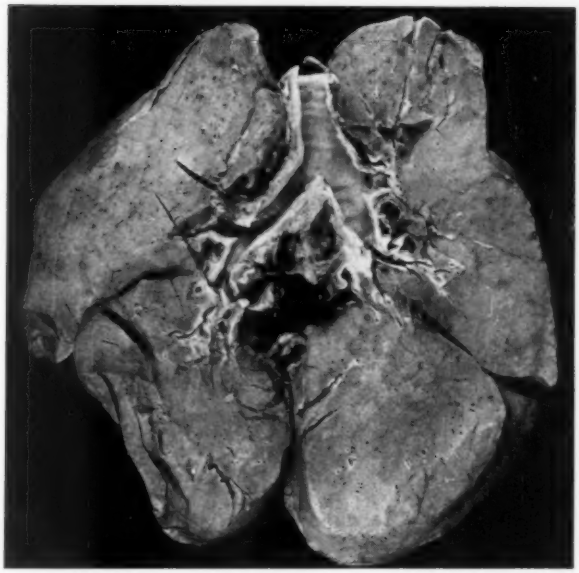

FIG. 2. The gross aspect of isolated serofibrinous pneumonia in influenza (BCH A-17175). Similar to *Case 1*.

Microscopically, the tracheobronchial tree revealed a severe necrotizing and membranous inflammation in which remnants of mucosa showed squamous metaplasia that sometimes extended into mucous glands and ducts. The lungs were only edematous and congested, and without evidence of infection, even in the smaller bronchioles. No bacteriologic studies were done.

Summary of Pathologic Findings

A summary of 17 fatalities associated with 1957 influenza is presented in table 1.[2] Three patterns of tissue change involving the tracheobronchial tree and lungs emerge, and these are illustrated by the case summaries above. The first, indicated by *Case 1*, consists of a necrotizing tracheobronchitis and bronchiolitis associated with a serofibrinous pneumonia, in which the alveoli are lined by hyalin membranes and hyperplastic epithelium. The predominant cell type is a large mononuclear (figures 2–5). The second, illustrated by *Case 2*, and probably an extension of the first, is characterized by widespread tissue destruction related to bacterial invasion. Here suppuration and bacterial colonization in the extreme are noted in isolated peribronchial confines as well as in broader confluences. Arterial walls are destroyed and lumina thrombosed so that the original insult is compounded by the death of tissue secondary to circulatory obstruction. Large abscesses and pulmonary hemorrhage are the expected sequels. In many areas of the lung, frankly atypical proliferation of squamous epithelium may be found (figures 6–9). The third reaction pattern, described in *Case 3*, is demonstrated by 2 older patients, both of whom had a prior history of congestive heart failure. The changes are limited to the upper tracheobronchial tree and are indistinguishable from the prior cases. These changes consist of a necrotizing and membranous inflammation with marked focal squamous metaplasia of the residual mucosa and mucous glands. No alterations are noted in the bronchioles, and the lung substance shows only edema and congestion compatible with the existence of cardiac insufficiency (figures 10–12).

The majority of the fatalities exhibited pulmonary changes that were mixtures and varia-

[2] A portion of the data on the cases indicated has been presented at a meeting of the American Federation for Clinical Research, Atlantic City, New Jersey, May 4, 1958, by Drs. F. McCrumb and P. Guerin of the University of Maryland School of Medicine.

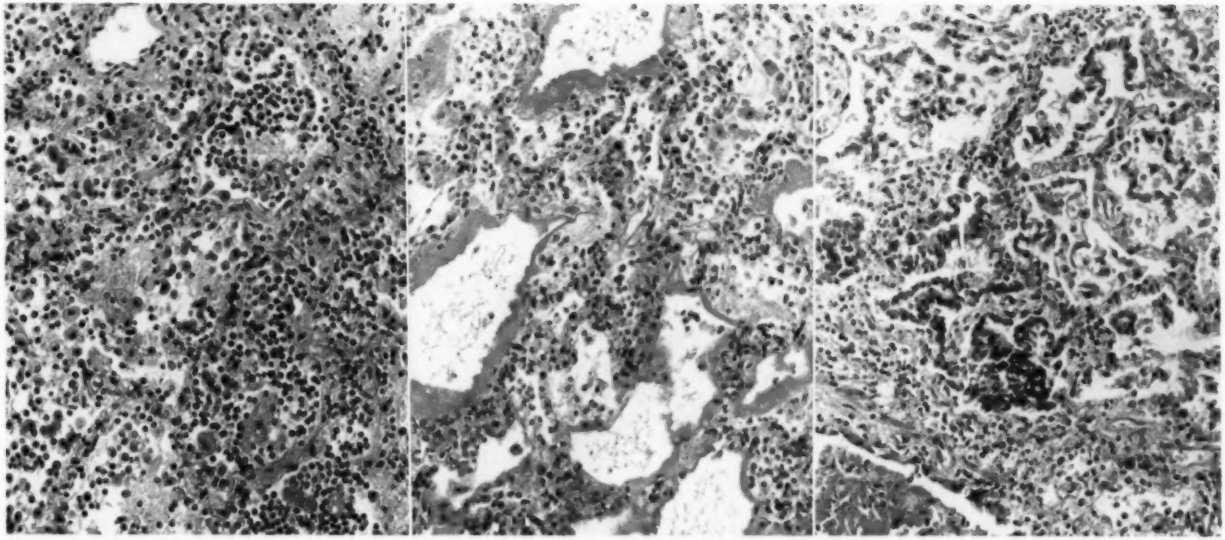

FIG. 3. (Left). Predominantly mononuclear alveolar infiltration in influenzal serofibrinous pneumonia (JHH A-27264). (× 250)
FIG. 4 (Center). Prominent alveolar hyalin membrane formation and interstitial mononuclear infiltration in influenzal serofibrinous pneumonia (JHH A-27264). (× 250)
FIG. 5 (Right). Alveolar epithelization in influenzal pneumonia (JHH A-27264). (× 250)

FIG. 6. Necrotizing pseudomembranous tracheobronchitis and suppurative lobular pneumonia in influenza (BCH A-16704).

tions of the types delineated above. Only the presence of necrotizing tracheobronchitis with focal squamous metaplasia of the epithelium was noted uniformly. Sometimes this involvement of the tracheobronchial tree was relatively inconspicuous or even unapparent to gross inspection but, microscopically, the changes were nonetheless characteristic.

The Relation of the Pathology to Bacterial and Viral Studies

It is obvious that important gaps exist in the cultural data. Failure to appreciate the possible existence of an influenzal infection and insufficient sampling may have been in part responsible for this. Influenza virus isolation was successful in six samples of pulmonary tissue taken from different hemorrhagic as well as frankly suppurative pneumonias. Unfortunately, no isolation studies were performed on material from cases of isolated tracheobronchitis. Presumptive serologic evidence of influenzal infection was obtained in 3 patients in whom an uncomplicated serofibrinous pneumonia existed.

Most samples of pulmonary tissue yielded *Staphylococcus pyogenes*, although it should be noted that one culture was sterile (*Patient R. K.*) when positive serologic evidence of influenzal infection was obtained. It is also of interest that pneumococci were twice isolated in proved cases of influenza when no frankly suppurative pneumonia existed.

DISCUSSION

Detailed study of 17 fatalities associated with the 1957 influenza epidemic in Baltimore has proved fruitful because proof of the existence of influenzal viral infection has been obtained in 9 patients. The information gained by study of the pathologic changes in the tracheobronchial tree and lungs corroborates what has long been known through the complete accounts of the 1918 influenza epidemic (13) and more recent smaller outbreaks (14, 15). Despite the extensive pulmonary inflammation induced by bacterial superinfection in numerous patients previously described and in some now reported, the morphology of the lesions of the tracheobronchial

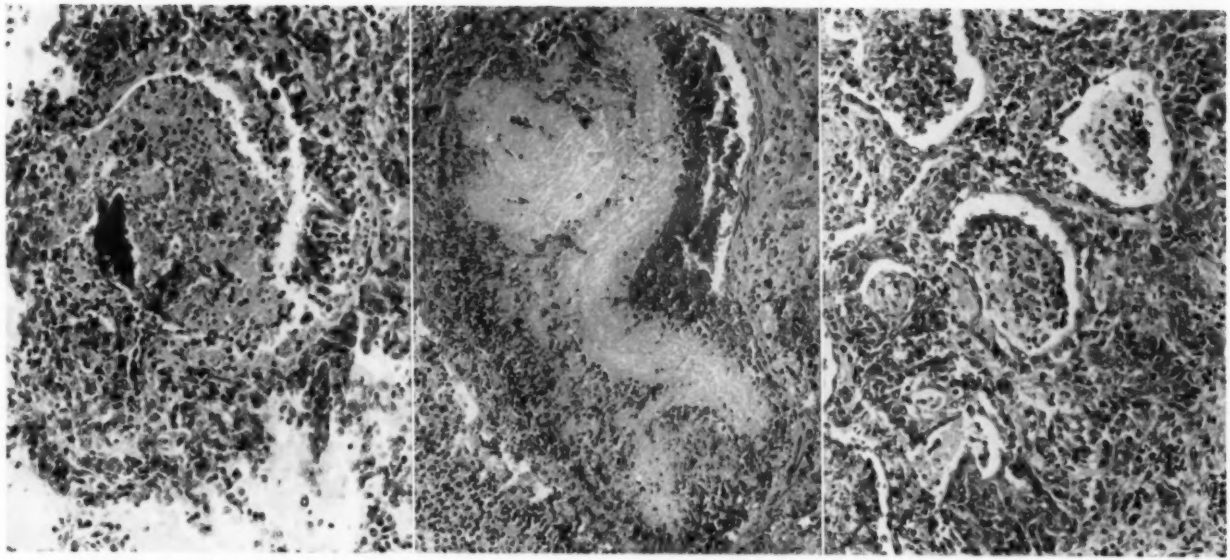

FIG. 7 (Left). Necrotizing bronchiolitis in influenza showing luminal plug and bacterial colonization (Staphylococcus) (BCH A-16704).
FIG. 8 (Center). Necrotizing vasculitis and thrombosis in suppurative pneumonia (influenzal) (BCH A-16704). (× 250)
FIG. 9 (Right). Focal proliferation of squamous epithelium in pulmonary parenchyma. Suppurative pneumonia (influenzal) (BCH A-16704). (× 250)

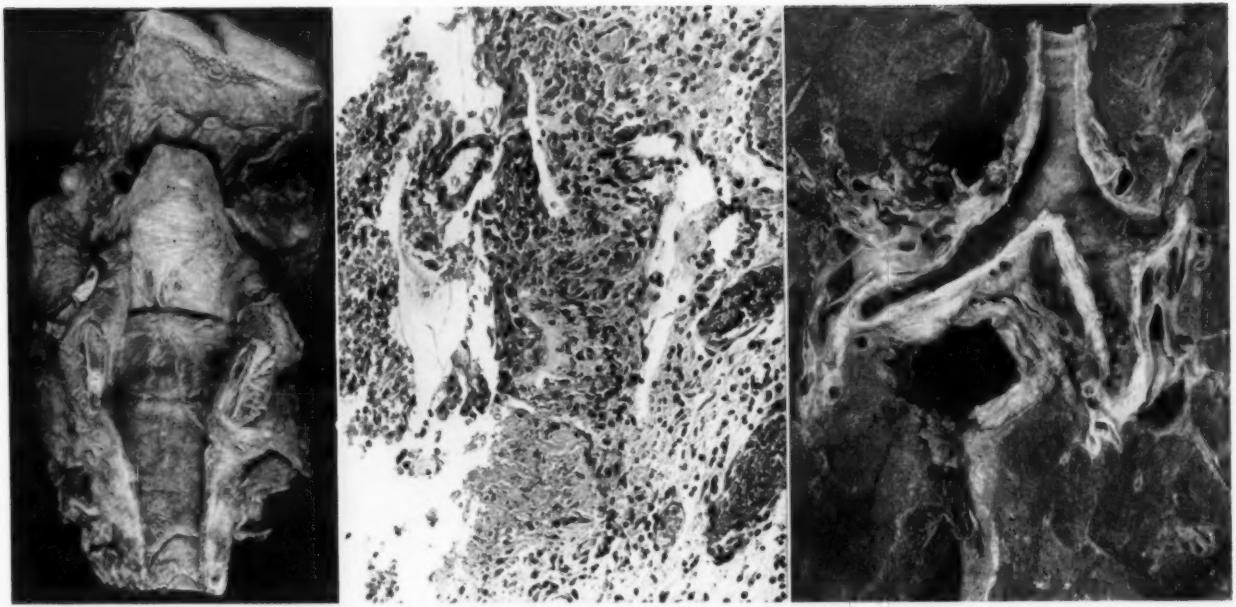

FIG. 10 (Left). Isolated necrotizing tracheobronchitis in influenza (BCH A-16841).
FIG. 11 (Center). Squamous cell regeneration in necrotizing tracheobronchitis (influenzal) (BCH A-16841). (× 250)
FIG. 12 (Right). Bronchial obstruction by exudate in influenzal tracheobronchitis. Note the absence of parenchymatous disease (BCH A-16841).

tree remains unique and distinctive as a feature in influenza.

It is particularly interesting that *Patient R. K.* demonstrated that an influenzal viral infection existed without detectable secondary bacterial invasion of the lung. The absence of cultural data in *Patients W. B. and M. B.*, who died with isolated tracheobronchitis, has delayed the forging of another link in the chain of evidence that influenzal viral infection alone may indeed provoke the changes observed. These cases may have been "pure" examples of influenzal viral infection, and it is to be noted that no bronchiolitis existed. Influenzal infection may be clinically occult in patients with existing cardiovascular liabilities (*Patients W. B. and M. B.*), and the meager evidence suggests that the fatal outcome may be more rapid, setting the stage for a future crucial opportunity to settle the controversy over the primacy of the virus or the bacterium in the development of the lesions.

The present experience also emphasizes a need for a more organized approach to the problem of influenza virus isolation from affected as well as unaffected tissues, in the presence and absence of known or suspected influenzal infection. The relation of the virus to changes recorded in expermental infections of ferrets and mice is known. That the serofibrinous outpouring into the alveoli associated with hyalin membranes, alveolar epithelization, and mononuclear infiltration is also an effect of the influenza virus is again suggested by *Patient R. K.*, who was bacterially sterile. It is significant that in the presence of a marked serofibrinous pneumonia, whether complicated by bacterial superinfection or not, demonstrations of influenzal viral infection were successful when attempted.

In regard to the type of bacterial infection in complicated and fatal cases, staphylococci stand pre-eminent. It has been postulated that staphylococci may produce the specific train of bronchopulmonary changes encountered in this and other studies, but there is no unassailable evidence for it.

Summary

The pulmonary pathology has been described in seventeen fatalities associated with the 1957 influenza epidemic. Evidence of influenzal infection was established in 6 patients by viral isolation from the lung, and in 4 patients by antibody studies. The lungs of all of the patients showed a characteristic necrotizing tracheobronchitis with foci of squamous metaplasia in the mucosa which is considered a distinctive feature of influenzal viral infection. Two patients exhibited isolated tracheobronchitis without a complicating pneumonia.

The inportance of *Staphylococcus aureus* as a predominant superinfecting organism in fatal cases is indicated.

Sumario

La Histopatología de la Influenza de 1957 (Asiática)

Se describe la histopatología pulmonar en 17 casos letales asociados con la epidemia de influenza asiática en 1957. Se obtuvieron pruebas de la infección gripal en 6 enfermos por medio del aislamiento del virus de los pulmones y en 4 por estudios de los anticuerpos de la hemaglutinación. Los pulmones de todos los enfermos mostraron una típica traqueobronquitis necrotizante con focos de metaplasia escamosa en la mucosa, lo cual se considera como característica distintiva de la infección por influenza viral. Dos enfermos manifestaron solamente traqueobronquitis sin neumonía como complicación.

Se señala la importancia en los casos letales del *Staphylococcus aureus* como predominante microbio superinfectante.

Resume

La pathologie de la grippe de 1957 (asiatique)

La pathologie pulmonaire est décrite dans 17 cas mortels de l'épidémie de grippe asiatique de 1957. Des preuves de l'infection grippale ont été établies chez 6 malades par l'isolement du virus dans les poumons et chez 4 malades par l'hémagglutination des anticorps. Les poumons de tous les malades présentaient une trachéo-bronchite nécrosante caractéristique avec foyers de métaplasie squameuse dans la muqueuse, ce qui est considéré comme un caractère distinctif de l'infection grippale d'origine virale. Chez 2 malades il y avait une trachéo-bronchite isolée sans pneumonie secondaire.

L'importance dans les cas mortels du *Staphylococcus aureus* comme germe surinfectant prédominant est indiquée.

Addendum

After the present report had been accepted for publication, an exhaustive study, largely clinical and correlative, of the pulmonary complications of influenza appeared in the *Journal of Clinical Investigation* (16). The writers showed with particular clarity the liability of associated cardiovascu-

lar disease, especially rheumatic heart disease, in their patients. In contrast to these findings, no evidence of rheumatic heart disease was observed in the 17 fatalities reported in the present paper, although 3 patients, all more than fifty years of age, had been previously treated for other cardiovascular disease. The importance of underlying cardiovascular disease in these few cases is emphasized by the observations herein recorded. The fact that 2 of these 3 fatalities exhibited isolated influenzal tracheobronchitis alone might suggest the possibility of a toxic factor adversely influencing hemodynamics, but the additional mechanical burden of obstructed airways probably provides a simpler and more persuasive explanation for the manner of death.

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THE HELIUM-DILUTION METHOD IN DETERMINATION OF VENTILATORY DISTURBANCES[1]

A New Method for Data Treatment

ERIK ORINIUS AND INGVAR STÅHLE

(Received for publication July 29, 1958)

Introduction

The functional residual capacity is often determined routinely by means of the helium-dilution method. In pulmonary disease it is also useful to find out if there is any major unevenness in the distribution of the ventilation. Due to the insufficiently rapid response of the apparatus, an uneven ventilation of minor degree cannot be determined with a helium catharometer of moderate sensitivity; faster instruments are required, for instance, nitrogen analyzers. Indeed, these have shown that even in healthy subjects the ventilation is slightly uneven. However, it may be so uneven (notably in emphysematous subjects) as to lend itself to determination with a helium catharometer of moderate sensitivity. From the clinical standpoint, moreover, it suffices to detect only pronounced cases of uneven ventilation.

Numerous suggestions have been made for the analysis of data in studies of uneven ventilation. Becklake (1), in an investigation in 1952 of healthy and emphysematous subjects, compared three earlier methods with a new one, and Buxton and D'Silva (2) reported a further method in 1956. The time required for indicator gas equilibrium between spirograph and lungs with a closed-circuit technique (as in the washing out of the lungs in an open-circuit system) is a function of the respiration rate, tidal volume, dead space, functional residual capacity, and (with the latter system) the spirograph volume. If a method of analyzing data is to be theoretically attractive, it should therefore include all of these factors as independent variables. Of the above-mentioned methods, only that of Bates and Christie (3) satisfies this desideratum. With their method, the calculated number of breaths required for 90 per cent dilution is taken in relation to the observed number. However, the method has been considered laborious for routine use.

[1] From the Pulmonary Physiology Laboratory, Kolmardssanatoriet, Sweden.

The aim of the present investigation has been to develop a method with a sound theoretical basis and convenient in practice.

Theoretical

For re-breathing in a closed-circuit system with helium as indicator gas, the following equation can be deduced:

$$\frac{He_t}{He_0} = \frac{S}{S + V_L}\left[1 + \frac{V_L}{S}\left(\frac{1 - \frac{V_A}{S}}{1 + \frac{V_A}{V_L}}\right)^{\frac{f \cdot t}{60}}\right]$$

where He_t and He_0 are the helium concentrations in the spirograph at the times t and 0 seconds, respectively; S is the spirograph volume in liters; V_L the functional residual capacity in liters; $V_A = V_T - V_D$ the alveolar ventilation per breath, i.e., the tidal volume less the dead space, all in liters; and f the respiration rate per minute. The equation is deduced on the supposition that the admixture in both lungs and in the spirograph proceeds rapidly enough to be completed before the next expiration or inspiration, as the case may be. Rauwerda (4) has presented evidence that this is the process in the lungs. With respect to the spirograph, the supposition is approximately fulfilled. From the above equation the time required for the helium concentration to complete 63 per cent of its total fall in a dilution test is obtained:

$$t_{0\text{-}63\text{calc.}} = \frac{60}{f \cdot \ln \frac{1 + \frac{V_A}{V_L}}{1 - \frac{V_A}{S}}}$$

which expression is too time-consuming for routine use. It can be approximated, however, to a more serviceable form, viz:

$$t_{0\text{-}63\text{calc.}} \simeq \frac{60}{\dot{V}_A\left(\frac{1}{V_L} + \frac{1}{S}\right)}$$

where $\dot{V}_A = V_A \cdot f$. Analysis with this equation can be further facilitated by a simple type of nomogram.

Apparatus

The spirograph employed in this investigation was of Knipping type, modified to a volume of 4.00 liters, excluding the bellows. The carbon dioxide was absorbed with a dry filter. The fan was adjusted to a capacity of about 50 liters per minute, and over it the catharometer circuit was shunted. The circuit was arranged so as to include passage of the gas through the water bottle filled with glass balls for its saturation; the gas then entered the catharometer and, upon leaving the catharometer, passed through a gas flow meter for ensuring a constant flow in the catharometer circuit. The temperature was continuously checked in both the spirograph and the catharometer circuit.

The helium gas mixture was prepared by adding to the spirograph, flushed with air, four parts of helium and one part of oxygen to a helium concentration of about 13 per cent. The spirograph, including the bellows, then contained 5.2 liters. During the subsequent re-breathing, an amount of oxygen equivalent to that consumed was supplied to the system, so that the respiratory curve had a horizontal course and the volume of the system was constant. If any subject was not connected properly with respect to his resting expiratory level, a correction was made in the functional residual capacity. During re-breathing each helium fall of 0.2 per cent was recorded on the spirogram manually with the aid of an electromagnetic device.

Procedure

The functional residual capacity and dilution time were obtained from the same measurement. In addition to the helium-dilution test the sub-

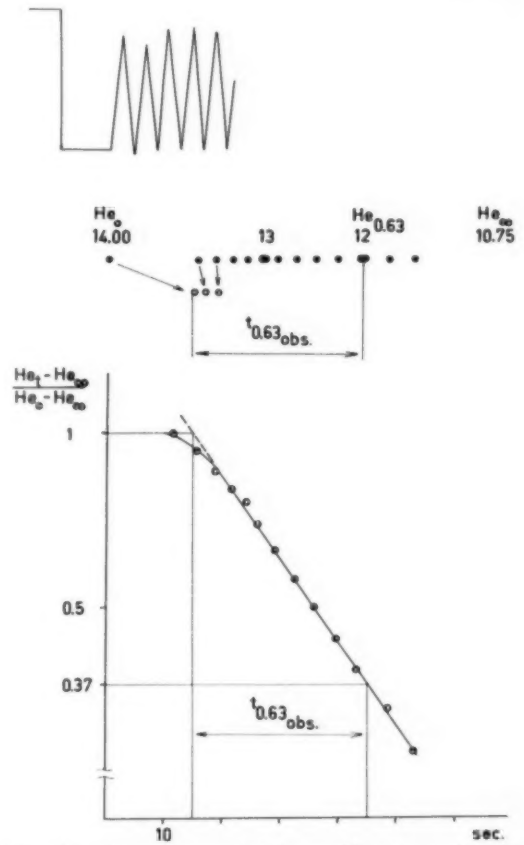

FIG. 1. *Upper half:* Drawing of a spirogram with its markings for each helium fall of 0.2 per cent during re-breathing. The approximate extrapolation in the marking series is shown with arrows. *Lower half:* An exact extrapolation in a logarithmic/linear system is illustrated.

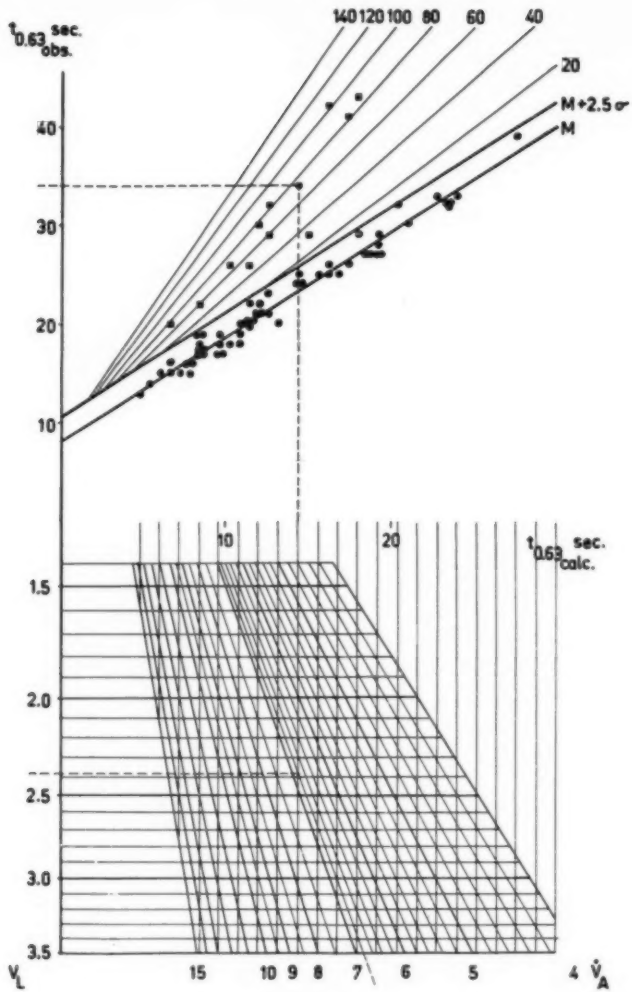

FIG. 2. *Upper half:* $t_{0.63_{obs.}}$ in seconds, plotted against $t_{0.63_{calc.}}$ in seconds for normal (●) and emphysematous subjects (■). The regression line of the normal subjects (M) and its upper limit ($M + 2.5\sigma$) are marked. The lines 20 to 140 represent per cent of helium-dilution delay (HDD) relatively calculated dilution time. *Lower half:* Nomogram for calculation of $t_{0.63_{calc.}}$, based on a volume of the spirograph of 5.2 liters. An example of calculation is marked (— — —): $\dot{V}_A$ = 6.8 liters/minute, V_L = 2.38 liters, $t_{0.63_{obs.}}$ = 34 seconds. Pathologic uneven ventilation. HDD = 70 per cent.

jects underwent an ordinary spirometric examination for the purpose, among other things, of determining the relative residual volume, the maximal breathing capacity, and the one-second vital capacity. The measurements were performed with the subjects supine. No efforts were made to obtain basal conditions.

Methods

It was found that the optimal range of accuracy for the dilution time corresponded to 60 to 80 per cent of completed dilution with this apparatus; and in this range 63 per cent was taken as a comparative level, since it was mathe-

matically advantageous to some degree. The nearer to 100 per cent the comparative level is set, the greater will be the manifestation in time of a difference between the actual and calculated dilution rates. But the closer the dilution approaches 100 per cent, the more slowly does it proceed and the greater is the time error due to that change in the calibration of the helium instrument which, with this apparatus, is unavoidable during the fall in concentration. It was, therefore, after mathematical assessment of these factors that the optimal accuracy was found to lie between 60 and 80 per cent, varying in different cases.

The time for 63 per cent dilution was adjusted for the initial delay but not for the continuous lag. Adjustment for the former is done by extrapolation to $He_t - He_\infty/He_0 - He_\infty = 1$. Theoretically this extrapolation, if it is to be done linearly, must be undertaken in a logarithmic/linear system; although it can be done, with very little error, within the line of markings on the spirogram (figure 1).

The average respiration rate and tidal volume were determined from the spirogram for the first ten, fifteen, or twenty seconds, depending on which of them best approximated the actual dilution time. The alveolar ventilation was calculated on the basis of a dead space of 120 cc. in males and 100 cc. in females (5, 6). Finally, the functional residual capacity was determined also. All volumes were adjusted to ambient pressure, saturated with water vapor.

The dilution time, obtained as described above, was then plotted against the time calculated with the aid of nomograms (figure 2).

Material

A normal series for the method was collected by measurements in 58 volunteers (mainly hospital employees), 31 males fifteen to thirty-nine years of age, and 27 females eighteen to thirty-one years old. None of them had a history of lung disease, and all of them were examined by roentgenography one to four months earlier, when the findings were negative.

The method was then tested in a series of 12 cases of emphysema. The criteria for the diagnosis were:

1. Etiologic factor, such as a long history of chronic bronchitis or bronchial asthma.
2. Roentgenographic factors: horizontal costae, broad intercostal spaces, depressed diaphragms, and sparse and thin pulmonary vessels.
3. Pathologic results of lung function determinations: ratio of residual volume to total lung capacity, one-second vital capacity, and maximal breathing capacity (table 1).

Results

One measurement was made in each subject. In the control series, the values in 2 cases greatly differed from the others. On redetermination, however, the values were consistent with the remainder, and the original ones were therefore assumed to have been due to measuring errors. The observed dilution times were, without exception, very closely correlated with the calculated ones (figure 2).

The male and female subjects were uniformly distributed relative to the mean dilution time for the control series.

The standard error of a single determination, calculated as the deviation from the mean of the observed dilution time at the relevant calculated

TABLE 1

Pulmonary Function Data of the 12 Cases of Emphysema (Supine Position)

Patient's Number	Sex	Age (years)	Height (cm.)	Weight (kg.)	Vital Capacity (liter)	Functional Residual Capacity (liter)	Residual Capacity (liter)	Total Lung Capacity (liter)	Functional Residual Capacity/Total Lung Capacity (per cent)	Residual Volume/Total Lung Capacity (per cent)	One-Second Vital Capacity (per cent)	Maximal Breathing Capacity (liter)	Helium Dilution Delay (per cent)
B 1281	M	64	186	69	2.4	4.6	3.7	6.1	75	69	48	25	80
B 2559	F	55	165	75	1.6	2.4	2.1	3.7	64	57	54	26	80
B 2447	M	63	170	67	3.6	2.8	2.2	5.8	47	38	69	60	50
B 2331	M	55	170	89	3.3	2.7	2.2	5.4	49	40	66	52	80
B 2600	M	58	175	82	3.0	2.9	2.4	5.4	54	45	71	60	50
B 2649	M	55	172	71	1.9	3.7	3.1	5.0	74	62	49	25	70
B 2555	F	65	160	45	1.2	1.5	1.2	2.4	63	56	74	29	70
B 2614	M	69	178	81	2.9	3.5	2.9	5.8	60	50	30	27	30
B 2236	M	66	181	71	3.3	3.8	3.0	6.3	61	48	51	45	60
B 2508	M	65	162	71	1.9	3.3	2.7	4.6	72	59	44	22	80
B 2644	M	54	183	60	2.2	3.1	2.6	4.9	64	46	45	39	80
B 2440	M	51	172	64	2.3	3.4	2.5	4.8	71	51	33	22	80

dilution time, was 0.8 second. This was based on duplicate measurements in 11 healthy subjects.

The variation in the regression line was 1.0 second. Taking the mean + 2.5 · the standard deviation as the upper limit, the equation for this will be:

$$t_{0.63\text{obs.}} = 1.05 \cdot t_{0.63\text{calc.}} + 10.8$$

All of the 12 patients with emphysema were well beyond this limit.

In order to obtain a quantitative result over and above the qualitative outcome, in the pathologic cases the difference between the observed and the corresponding normal dilution time can be taken in relation to the calculated time. This ratio, expressed as a percentage, is designated as the helium dilution delay, as may be seen in figure 2. In view of the error of the method, it should not be given with a greater accuracy than 10 per cent.

Discussion

Determination of the actual dilution time is complicated and unnecessary. To be justified in plotting the observed against the calculated dilution time, it suffices merely to ascertain that the factors which distinguish the former from the latter are virtually constant and, if not, to correct for systematic variations.

The lag in the through-circuit time for the spirograph and catharometer, the acceleration delay, and the continuous lag of the galvanometer are the factors that separate the observed, uncorrected dilution time from the actual one. The last-named of these factors was investigated in a few experiments and, at 63 per cent dilution, was found to be almost constant at various dilution rates relevant here. The through-circuit time plus the acceleration delay, on the other hand, showed individual variations up to 7 seconds. A correction for this was accordingly inserted by extrapolation to $He_t - He_\infty/He_0 - He_\infty = 1$.

Here, however, the duration of the first inspiration is also eliminated. Although this duration is included in the calculated dilution time, its elimination would not matter if it were identical in all subjects; but, in point of fact, it varies, of course, with the respiration rate. In resting pulmonary function tests, respiration rates lower than 9 and higher than 22 per minute are seldom if ever found. Within this range the differences in duration of an inspiration are so small that a correction for them can be ignored, along with the errors of measurement and extrapolation.

The calculated dilution time is dependent on the respiration rate, tidal volume, functional residual capacity, and spirograph volume (which are all measured) and on the dead space (which is assumed) and the approximation. Determinations of the respiration rate, tidal volume, functional residual capacity, and spirograph volume are not associated with any major systematic errors; but the dead space is; for a discrepancy between the assumed mean dead space in the controls and the actual one will be of relatively greater significance with smaller than with larger tidal volumes. The slope of the regression line in the control determinations will therefore depend on the accuracy of the postulated mean dead space, for the lower tidal volumes do not coincide to the same extent with each of the dilution times; they are mostly associated with the longer ones. The value of 1.05 for the regression coefficient might then, by comparison with the mathematically expected value of 1, suggest that the postulated mean dead space was somewhat low.

Theoretically, however, the coefficient is greater than 1 for two reasons: first, the fact that admixture in the spirograph is not quite complete; and second, the somewhat uneven ventilation even in healthy subjects. The dilution delay in these subjects, provided it varies with the calculated dilution time, tends to raise the regression coefficient. Due to the incomplete admixture in the spirograph, the subjects inhale a more concentrated helium gas mixture; hence, dilution occurs faster than expected. The admixture is least complete at high respiration rates. In this way the shorter dilution times accompanying higher respiration rates will be too short, thus elevating the regression coefficient. The accuracy of the assumed mean dead space cannot, therefore, be estimated on the basis of the observed regression coefficient.

The individual variations about the mean of the dead space could probably amount to more than 10 cc. Even though a variation of 10 cc. in the dead space seldom means as much as a half-second variation in the calculated dilution time, the influence of the individual variations may accordingly amount to two to three seconds plus or minus in the calculated dilution time, although it is usually far less.

The error introduced through the approximation is fairly constant, for in this series it amounts to between minus one-half and minus one and one-half seconds in the calculated dilution time. It can be studied when expressed as a correction factor for the calculated dilution time

$$K \simeq 1 + \frac{V_A}{z}\left(\frac{1}{V_L} - \frac{1}{S}\right)$$

From this it is evident that the error will not be appreciable until high V_A values are reached. At such values, however, the dilution time is generally short; hence the error remains fairly constant throughout the range.

On the basis of

$$\frac{He_t}{He_0} = \frac{S}{S+V_L}\left[1 + \frac{V_L}{S}\left(\frac{1-\frac{V_A}{S}}{1+\frac{V_A}{V_L}}\right)^{\frac{f\cdot t}{60}}\right] \qquad 1.$$

and the dilution equation

$$He_0 S = He_\infty (S + V_L) \qquad 2.$$

and the definition

$$He_{0.63\text{calc.}} = He_0 - 0.63(He_0 - He_\infty) \simeq$$

$$\simeq He_0 - \left(1 - \frac{1}{e}\right)(He_0 - He_\infty) = \qquad 3.$$

$$= He_\infty + \frac{1}{e}(He_0 - He_\infty)$$

there is obtained, by combination of 1 and 3:

$$\frac{He_\infty}{He_0} + \frac{1}{e} - \frac{1}{e}\cdot\frac{He_\infty}{He_0} =$$

$$= \frac{S}{S+V_L} + \frac{V_L}{S+V_L}\left(\frac{1-\frac{V_A}{S}}{1+\frac{V_A}{V_L}}\right)^{\frac{f\cdot t_{0.63\text{calc}}}{60}} \qquad 4.$$

From dilution equation 2 is obtained:

$$\frac{He_\infty}{He_0} = \frac{S}{S+V_L}$$

which is inserted in 4, out of which emerges, after simplification:

$$\left(\frac{1-\frac{V_A}{S}}{1+\frac{V_A}{V_L}}\right)^{\frac{f\cdot t_{0.63\text{calc.}}}{60}} = \frac{1}{e}$$

Hence,

$$t_{0.63\text{calc.}} = -\frac{60}{f\cdot\ln\dfrac{1-\frac{V_A}{S}}{1+\frac{V_A}{V_L}}}$$

Approximations are done in accordance with traditional formulas for calculation with small figures:

$$t_{0.63\text{calc.}} = \frac{60}{f\cdot\ln\dfrac{1+\frac{V_A}{V_L}}{1-\frac{V_A}{S}}} \simeq \frac{60}{f\cdot\ln\left(1+\frac{V_A}{V_L}\right)\left(1+\frac{V_A}{S}\right)} =$$

$$= \frac{60}{f\left[\ln\left(1+\frac{V_A}{V_L}\right)+\ln\left(1+\frac{V_A}{S}\right)\right]} \simeq$$

$$\simeq \frac{60}{f\left(\frac{V_A}{V_L}+\frac{V_A}{S}\right)} = \frac{60}{f V_A\left(\frac{1}{V_L}+\frac{1}{S}\right)}$$

An expression for the error of approximation can be computed in the form of a correction factor, with due regard to second degree terms:

$$t_{0.63\text{calc.}} \simeq \frac{60}{f\cdot\ln\left(1+\frac{V_A}{V_L}\right)\cdot\left[1+\frac{V_A}{S}+\left(\frac{V_A}{S}\right)^2\right]} =$$

$$= \frac{60}{f\left\{\ln\left(1+\frac{V_A}{V_L}\right)+\ln\left[1+\frac{V_A}{S}+\left(\frac{V_A}{S}\right)^2\right]\right\}} \simeq$$

$$\simeq \frac{60}{f\left\{\frac{V_A}{V_L}-\frac{1}{2}\left(\frac{V_A}{V_L}\right)^2+\frac{V_A}{S}+\left(\frac{V_A}{S}\right)^2-\frac{1}{2}\left[\frac{V_A}{S}+\left(\frac{V_A}{S}\right)^2\right]^2\right\}} \simeq$$

$$\simeq \frac{60}{f\left[\frac{V_A}{V_L}+\frac{V_A}{S}-\frac{1}{2}\left(\frac{V_A}{V_L}\right)^2+\frac{1}{2}\left(\frac{V_A}{S}\right)^2\right]} =$$

$$= \frac{60}{f\left(\frac{V_A}{V_L}+\frac{V_A}{S}\right)\cdot\left[1-\frac{1}{2}\left(\frac{V_A}{V_L}-\frac{V_A}{S}\right)\right]} \simeq$$

$$\simeq t_{0.63\text{calc.}}\cdot\left[1+\frac{V_A}{2}\left(\frac{1}{V_L}-\frac{1}{S}\right)\right]$$

Summary

A method for treatment of data in studies of the unevenness in the ventilation by means of the helium-dilution method is described and discussed. A normal series for the method is reported.

The method was tested in 12 verified cases of emphysema, in all of which the results differed appreciably from those in the control series. The selectivity and convenience of the method make it suitable for clinical use, and experience from its use in a large clinical series will be reported subsequently.

Sumario

El Método de la Dilución del Helio en la Determinación de los Trastornos de la Ventilación: Nuevo Método para el Tratamiento de los Datos

Describese y discútese un método destinado al tratamiento de los datos en los estudios de la dilución del helio. Preséntase una serie nomal para el método.

El método fué ensayado en 12 casos comprobados de enfisema, en todos los cuales los resultados discreparon apreciablemente de los obtenidos en la serie testigo. La selectividad y la conveniencia del método lo hacen apropiado para empleo clínico y más adelante se presentarán los datos derivados de su uso en una numerosa serie clínica.

Resume

La méthode de dilution dans l'hélium dans la détermination des troubles de la ventilation. Une méthode nouvelle pour le traitement des données

Une méthode pour le traitement des données dans les études sur l'inégalité de la ventilation au moyen de la méthode de dilution dans l'hélium est décrite et commentée. Une série normale selon cette méthode est rapportée.

La méthode a été testée dans 12 cas d'emphysème vérifié, dans tous ces cas les résultats différaient notablement de ceux des séries de contrôle. La sélectivité et la commodité de la méthode la rend adaptable à l'usage clinique et l'expérience de son emploi dans une série clinique importante sera rapportée ultérieurement.

Acknowledgment

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RESPIRATORY FUNCTION TESTS[1, 2]

Normal Values at Median Altitudes and the Prediction of Normal Results

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INTRODUCTION

A knowledge of the range of normal values is essential for the interpretation of biologic tests. In the case of respiratory function, test values vary with the age and body build of the subject (1–8) and with the technique of performing the test, while it is possible that the altitude at which the studies are made may also influence the results. Detailed studies (2, 3) from two sea-level laboratories have shown a relationship between the more commonly used lung function tests and body characteristics, thus providing normal prediction formulas for practical use at sea level. There are few data, however, on subjects resident at median altitudes, particularly on the subdivisions of lung volume, although it has been suggested on clinical grounds that vital capacity and functional residual capacity may be increased at these altitudes (9). It was the purpose of this study to examine lung function in subjects resident at a median altitude (5,760 feet above sea level) to see if there was any evidence that a reduced barometric pressure influenced any test values.

MATERIAL AND METHODS

Ninety-four subjects were tested, 44 males and 50 females. They included professional and clerical members of the hospital staff and their relatives. All were in good health at the time. None were in athletic training and none were engaged in manual labor, though all were gainfully employed. Apart from willingness to take part in the study, criteria for selection of subjects were: a freedom from major respiratory or cardiac illness or any general debilitating disease (past or present) and, in most subjects, a normal chest roentgenogram (this procedure was carried out in all of the older subjects and in most of the younger ones).[5] A remote single attack of pneumonia without complications and subsequent symptoms did not exclude the subject.

All tests were made with the subjects seated. Measurements of vital capacity, recording the maximal expired volume after a deep inspiration, expiratory reserve volume, and inspiratory capacity were made on a Knipping-type spirometer. Functional residual capacity was measured by the closed-circuit, constant-volume method of McMichael (10) using hydrogen as a test gas and incorporating previously described modifications (11). From the rate of equilibration of lung gas with the gas in the spirometer circuit, a mixing index was calculated (12). Values for residual volume were obtained by subtracting the expiratory reserve volume from the functional residual capacity. The maximal breathing capacity was measured on a Benedict-Roth spirometer fitted with smooth rubber tubing of 1-inch internal bore, from which a soda lime cannister and valves had been removed. It was subsequently shown that the resistance in this circuit was unduly high, and the frequency response was inadequate for the purpose (13). However, maximal breathing capacity results are included for comparison with those of other laboratories in which the same instrument had been employed. No set respiratory rate was followed, but subjects were urged to breathe as deeply and rapidly as possible.

A standard step test (14) was performed by each subject at a work rate of 300 kg. m./min., and values for resting ventilation, exercise and standardized ventilation, as well as for dyspneic index, were recorded (14). Arterial oxygen saturation was recorded on an oximeter[6] at rest, during exercise, and subsequently during the inhalation of pure oxygen.

All gas volumes were adjusted to body temperature, saturated and ambient pressure. Age was recorded to the nearest year; height was measured in centimeters, the subject standing in his socks, while weight was recorded to the nearest kilogram, and surface area was derived from du Bois' tables (15).

Derivation of formulas: The multiple correlation coefficient between any one lung function test

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[2] The work described in this paper includes material which has been submitted by one of us (H.I.G.) for a Ph.D. thesis to the University of Pretoria.

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[5] In 30 males and 27 females, chest roentgenograms were obtained and were normal.

[6] Waters Conley absolute reading oximeter.

and the four body characteristics (age, height, weight, and surface area) was calculated. The significance of each variable in this value was estimated from the t value for beta (16), and the least significant variables were then eliminated seriatim (*see* also table 5). For instance the multiple correlation coefficient of vital capacity in males with the four body variables studied was 0.866 and, after eliminating weight and surface area from the correlation coefficient, it remains 0.855, indicating that these two variables relate minimally to vital capacity. Prediction formulas for each measurement based on two or one variable were derived in this way.

To avoid repeated calculations, tables were drawn up in which the two variables concerned appeared on the x and y axes, respectively, and the predicted value in the relevant square.

Obviously, once three variables are included in a formula, graphic methods are very much more difficult to use. All formulas, therefore, were deliberately reduced to two and one variable, with no very great loss of accuracy (*see* table 5).

Results

The mean and standard deviation of the values for lung volume subdivisions are shown in table 1, which includes values obtained by other workers for the purposes of comparison (1, 3, 4, 6–8, 17–24). Some of these results are given at room temperature, not body temperature, saturated and ambient pressure (3, 21, 24). An average correction has been applied to these values to enable direct comparison, although it was realized that such a correction was not accurate. Similarly, when subjects were tested in the lying position (1, 4, 6), results have been adjusted by an amount appropriate for the effect of posture on test values (25). The difference between the mean value for any one test in this series and the value for the same test in another series was considered significant if P values were less than 0.05, using "Student's" t-test (26).

It will be seen that the present values differ in many instances significantly from those obtained by other workers. However, the question immediately arises to what extent these differences can be ascribed to differences of age and size, which are often considerable (table 2).

It was thought that one method of comparison which would allow for these differences would be to compare other workers' mean values with a predicted value, using prediction formulas based on the intercorrelations of test results and body characteristics found in the present series. However, this maneuver was only considered permissible if it could be shown that the relationship of various lung volume measurements to age and body characteristics was the same in the present series as has been found elsewhere. These relationships were accordingly examined.

Total correlation coefficients relating the subdivisions of lung volume and maximal breathing capacity to body characteristics were calculated for the present series from paired observations. These are shown in tables 3 and 4, which also show the values for similar correlations encountered in the literature. Comparison of the correlation coefficients of the present study with those reported by others was done using Fisher's z-transformation of the correlation coefficient (26), differences being considered significant where $P < 0.05$. It was found that the values obtained in this study were similar to those reported by all other workers with only two minor exceptions. The first of these was the ratio residual volume/total lung capacity $\times$ 100 per cent in male subjects, which was found by the present workers and by one other group (3) to increase with age, but was found to be independent of age by Whitfield and associates. The second exception concerned the relationship of residual volume to height in female subjects, found to be significant in the present study and in one other report (24), but reported not significant by Needham and co-workers (3). However, Needham and co-workers found that both vital capacity and total lung capacity correlated significantly with height. It seems, therefore, improbable that total lung capacity minus vital capacity or residual volume should not also relate to height. Since the correlation coefficients found in this series were for practical purposes no different from those reported elsewhere and the regression equations reported by other workers did not on inspection appear to be very different from those found here, it was believed that the method for comparison suggested above might be used. Accordingly, formulas for predicting these values from age and body characteristics were derived (table 5), using the method advised by Snedecor for four variables (16).

The mean values for the various subdivisions of the lung volume and maximal breathing capacity found elsewhere were then compared with values predicted from these formulas (shown in table 1) in an attempt to eliminate differences due to age and body build of the normal subjects used in the various studies. From inspection of the

TABLE 1

MEAN VALUES AND STANDARD DEVIATIONS OF VITAL CAPACITY, RESIDUAL VOLUME,

TABLE 1

MEAN VALUES AND STANDARD DEVIATIONS OF VITAL CAPACITY, RESIDUAL VOLUME, FUNCTIONAL RESIDUAL CAPACITY, RESIDUAL VOLUME/TOTAL LUNG CAPACITY × 100, AND MAXIMAL BREATHING CAPACITY OBTAINED IN PRESENT SERIES COMPARED WITH VALUES REPORTED BY OTHER WORKERS

Author	Reference Number	VC			RV			FRC			$\frac{RV}{TLC} \times 100$			MBC		
		Mean	SD	Predicted	Mean	SD	Predicted	Mean	SD	Predicted	Mean	SD	Predicted	Mean	SD	Predicted
Male Subjects																
													per cent			
Goldman and Becklake	—	4.44	0.876	4.48	2.04	0.482	2.01	3.65	0.738	3.43	31.9	7.33	32	137.6	38.66	138
Zwi et al.	17	4.67	0.579	4.45	1.87	0.457	2.03	3.47	0.692	3.44	29.7	8.19	32	156.8*	30.31	135
Needham et al.†	3	4.58	0.824	4.35	2.31*	0.572	1.84	3.66	0.747	3.33	33.8	7.40	31	133.0	26.38	137
Whitfield et al.†	24	4.24	0.917	4.31	1.747*	0.584	1.77	3.016*	0.773	3.35	30.2	8.50	30			
Baldwin et al.‡	1	4.18	0.749	4.33									31	108.3*	25.71	132
Kaltreider et al.‡	4	4.36	0.620	4.11	(1.52*)	0.410	1.97	(2.78*)	0.500	3.25	21.0*	5.58	33			
Astlett et al.	7	4.18	0.604	4.21	1.59*	0.500	1.71	2.92*	0.600	3.19	29.0	6.60	30			
Gilson and Hugh-Jones	18	4.77	0.759	4.50	2.12	0.523	1.85	3.70	0.675	3.46	30.7	6.95	30			
Greifenstein	19	3.48*	0.480	3.63	2.43*	0.500	2.17	3.44	0.740	3.30	40.9*	7.10	38	76.7*	14.40	95
Pemberton and Flanagan	7	4.03*	0.740	4.04									34			
Birath	20	5.08*	0.480	5.05	1.49*	0.230	1.79	3.18*	0.450	3.63	22.7*	2.50	27			
Hurtado and Boller‡	6	5.12*	0.590	5.11	(1.38*)	0.350	1.69	(3.15*)	0.500	3.60	21.0*	4.40	25			
Frank et al.†	21	5.32*	0.630	5.33												
Anderson et al.	22															
Marshall	23	5.05	0.876	5.27	1.20	0.746	1.74	3.54	1.130	3.74	19.3		25	149.1	45.25	
Female Subjects																
Goldman and Becklake	—	3.24	0.635	3.32	1.55	0.430	1.60	2.71	0.578	2.73	31.9	7.15	32	99.8	21.84	99
Needham et al.†	3	3.03	0.594	3.23	1.73	0.418	1.60	2.53	0.539	2.74	36.4	7.20	33	92.3	17.58	92
Whitfield et al.†	24	3.16	0.556	3.30	1.41	0.468	1.58	2.29*	0.510	2.85	32.2	7.90	32			
Baldwin et al.‡	1	3.12	0.509	3.30										85.6*	17.60	96
Greifenstein	19	2.34*	0.450	2.71	1.78	0.400	1.67	2.22*	0.530	2.44	41.9		38	68.8*	27.80	79
Birath	20	3.57*	0.440	3.53	1.22*	0.250	1.57	2.22*	0.490	2.81	25.5	2.80	30			
Frank et al.†	21	3.87*	0.520	3.73												
Anderson et al.	22															
Hurtado et al.	34	3.37	0.410	3.70	(1.45)	0.310	1.48	(2.75)	0.400	2.90	29.9	5.00	27	99.5	17.88	114
Marshall	23	2.83	0.581	3.39	1.32	0.370	1.23	2.23	0.581	2.65	31.8		28			

* Value differs significantly ($P < 0.05$) from present figure using Student's t-test.
† Average temperature correction applied.
‡ Correction made for fact that these estimations were done with subject lying.
( ) FRC measured by a technique known to give lower values.
VC—vital capacity
SD—standard deviation
RV—residual volume
FRC—functional residual capacity
TLC—total lung capacity
MBC—maximal breathing capacity

TABLE 2

BODY CHARACTERISTICS OF THE VARIOUS NORMAL SERIES STUDIED

Author	Sex	Number of Subjects	Age		Height		Weight		Surface area		Source of Material	Altitude above Sea Level in feet
			Years	SD†	Cm.	SD	Kg.	SD	M^2	SD		
Goldman and Becklake	M	44	44.3	16.60	173.9	7.22	74.6	11.80	1.89	0.160	Sedentary workers	5,760
Zwi *et al.*	M	30	45.3	8.65	174.4	5.26	73.5	10.07	1.88	0.130	Manual workers	5,760
Needham *et al.*	M	102	41.2	13.20	170.5*	6.34	68.8*	9.10	1.81*	0.140	Hospital staff and patients, factory workers	Sea level
Whitfield *et al.*	M	58	39.1	16.80	169.6*	7.62	61.5*	9.50			Students, staff, patients	600
Baldwin *et al.*	M	52	42.9	15.40	171.7	8.07	65.8*	9.17	1.79*	0.142	Convalescents	Sea level
Kaltreider *et al.*	M	50	48.2	6.40	170.5	7.20	70.8	12.10	1.82	0.167	Subjects gainfully employed	700
Astlett and McMichael	M	66	38.3*	9.60	167.1*	5.80	64.5*	8.50	1.71*	0.130	Coal miners	Sea level
Gilson and Hugh-Jones	M	40	39.5	11.10	171.8	6.87	67.0*	9.58			Manual workers	Sea level
Greifenstein	M	11	61.5	6.80	169.0	4.80	65.9	12.40	1.74	0.140	Hospital staff and patients	Sea level
Pemberton	M	428	52.3	9.72	171.4	6.64	76.5	11.20			Factory operatives	220
Birath	M	16	29.3*	5.57	176.5	4.89	73.8	9.13			Professional men	Sea level
Hurtado and Boller	M	50	22.9*	3.30	176.2	5.10	72.5	11.20	1.88	0.125	Students and staff	700
Frank and Mead	M	38	25.0*	5.00	178.0	8.00			1.91	0.180	Hospital staff	Sea level
Marshall	M	38	24.0*	6.99	176.6	14.95	71.1	13.73	1.85	0.259	Students and physicians	Sea level
Goldman and Becklake	F	50	38.5	16.30	160.9	6.40	62.3	7.92	1.65	0.138	Sedentary workers	5,760
Needham *et al.*	F	66	42.1	14.40	160.0	5.90	58.5*	9.70	1.60	0.140	Hospital staff, patients, factory workers, business women	Sea level
Whitfield *et al.*	F	31	38.2	17.00	160.3	8.40	53.1*	9.40			Students, staff, patients	600
Baldwin *et al.*	F	40	40.9	16.50	161.2	6.60	62.7	12.40	1.65	0.153	Convalescents	Sea level
Greifenstein	F	15	60.4	7.70	157.0	6.40	66.8	10.10	1.67	0.150	Hospital staff and patients	Sea level
Birath	F	19	29.9*	6.20	162.3	4.70	62.1	11.00			Professional women	Sea level
Frank and Mead	F	32	26.0	5.00	164.0	6.00			1.64	0.110	Hospital staff	Sea level
Hurtado *et al.*	F	50	23.1*	3.40	163.4*	4.20	57.2*	9.40	1.60	0.136	Students and nurses	700
Marshall	F	8	20.1*	4.57	156.4	8.52	51.3*	11.40	1.46	0.657	Students and physicians	Sea level

* Value differs significantly from that in the present series ($P < 0.05$ using Student's t-test).

† Standard deviation.

TABLE 3

CORRELATION COEFFICIENTS OF VITAL CAPACITY, RESIDUAL VOLUME, FUNCTIONAL RESIDUAL CAPACITY, RESIDUAL VOLUME/TOTAL LUNG CAPACITY × 100, AND MAXIMAL BREATHING CAPACITY WITH BODY CHARACTERISTICS

Comparison of Present Values with Those Obtained by Other Workers

Males	Present Series	Whitfield *et al.* (2)	Needham *et al.* (3)	Astlett *et al.* (7)	Baldwin *et al.* (1)	Kaltreider *et al.* (4)	Hurtado and Fray (5)	Pemberton (8)
Number of Cases	44	58	102	66	52	50	50	428
Vital capacity with								
Age	−0.668	−0.423	−0.74		−0.432			−0.47
Height	+0.618	+0.629	+0.58	+0.70	+0.485	+0.636	+0.545	+0.497
Weight	+0.318	+0.373	+0.36	+0.57	+0.227	+0.309	+0.138	+0.167
Surface area	+0.460		+0.48	+0.67	+0.431	+0.446	+0.279	
Residual volume with								
Age	+0.484	+0.346	+0.49	+0.49				
Height	+0.294	+0.168	+0.05	+0.05				
Weight	+0.075	+0.024	−0.17	−0.17				
Surface area	+0.216		−0.10	−0.10				
Functional residual capacity with								
Age	−0.085	+0.125				+0.030		
Height	+0.497	+0.326				+0.280		
Weight	+0.024	+0.103				−0.17		
Surface area	+0.186					−0.03		
$\frac{RV}{TLC} \times 100$ *with*								
Age	+0.761	−0.218*	+0.77					
Height	−0.111	+0.060	−0.28					
Weight	+0.018	−0.019	−0.34					
Surface area	−0.060		−0.35					
Maximal breathing capacity with								
Age	−0.785		−0.66		−0.63			
Height	+0.333		+0.41		+0.41			
Weight	+0.241		+0.28		+0.27			
Surface area	+0.306		+0.37		+0.36			

* Value differs significantly from that obtained in the present series ($P < 0.05$).

results it will be seen that many of the differences now disappear. For instance, for vital capacity in male subjects there are five other series (6, 7, 19–21) whose values differ significantly from the present findings, and for female subjects values significantly different from the present ones are reported in three studies (19–21). In all of these instances, however, the reported values agree closely with the predicted value; in other words, such differences as were observed appear to be explicable on differences of age and body characteristics.

Comparison of the functional residual capacity values for the present series with others reported in the literature (table 1) shows that in five other series (4, 6, 7, 20, 24) the values for males were significantly lower, and in three other series (19, 20, 24) the values for females were significantly lower. In two of these instances (4, 6) the technique used to make the measurement was a modification of the Christie method, which has been shown to give lower values than other methods, the reason being that no true equilibration is reached between spirometer and lungs, as circuit volume is continually decreasing in proportion to the subject's oxygen uptake (27). It is likely that this reason also accounts for the differences between the present values and those

TABLE 4

CORRELATION COEFFICIENTS OF VITAL CAPACITY, RESIDUAL VOLUME, FUNCTIONAL RESIDUAL CAPACITY RESIDUAL VOLUME/TOTAL LUNG CAPACITY × 100 AND MAXIMAL BREATHING CAPACITY WITH BODY CHARACTERISTICS

Comparison of Present Values with Those Obtained by Other Workers

Females	Present Series	Whitfield *et al.* (2)	Needham *et al.* (3)	Baldwin *et al.* (1)	Hurtado *et al.* (34)
Number of cases	50	31	66	40	50
Vital capacity with					
Age	−0.528	−0.37	−0.63	−0.59	
Height	+0.574	+0.336	+0.54	+0.501	+0.513
Weight	+0.022	+0.286	+0.14	+0.070	+0.260
Surface area	+0.266		+0.30	+0.260	+0.338
Residual volume with					
Age	+0.274	+0.239	+0.34		
Height	+0.422	+0.366	+0.00*		
Weight	+0.061	+0.053	−0.08		
Surface area	+0.201		−0.06		
Functional residual capacity with					
Age	−0.027	+0.050	−0.03		
Height	+0.521	+0.494	+0.18		
Weight	−0.264	+0.161	−0.26		
Surface area	+0.042		−0.15		
$\frac{RV}{TLC} \times 100$ *with*					
Age	+0.593		+0.66		
Height	+0.000		−0.30		
Weight	+0.156		−0.13		
Surface area	+0.068		−0.20		
Maximal breathing capacity with					
Age	−0.615		−0.62	−0.54	
Height	+0.403		+0.30	+0.460	
Weight	+0.162		+0.00	+0.09	
Surface area	+0.258		+0.24	+0.24	

* Value differs significantly from that obtained in the present series ($P < 0.05$).

of Birath (20). In three other instances (7, 19, 24), differences between results of other workers and the present series are much less striking when the value in question is compared with the predicted value; in other words, when allowance is made for differences in body size. It, therefore, seemed reasonable to conclude that there is no consistent difference between sea-level values and those from the present series.

The trends in residual volume values follow functional residual capacity values closely, those workers who used Christie-type circuits for measurement reporting lower values. In one study done at sea level (3), however, considerably higher figures were recorded which appeared to have nothing to do with differences in the age and size of the subject. It may be remembered, however, that the residual volume is usually measured as functional residual capacity minus expiratory reserve volume; in other words, is dependent on expiratory reserve volume, a measurement which may be influenced by effort on the patient's part and resistance in the measuring circuit. Thus, differences in residual volume not reflecting similar variations in functional residual capacity are probably of little significance.

The maximal breathing capacity values in this series are higher than any others reported in the literature except those of Anderson and associates (22) working at an altitude similar to that of Johannesburg, and those of Zwi and co-workers (17) working in Johannesburg. These differences are not eliminated when age and size are taken into account, and might have been more striking

TABLE 5

PREDICTION FORMULAS FOR SUBDIVISIONS OF LUNG VOLUME, MAXIMAL BREATHING CAPACITY, AND RESTING VENTILATION

Value to Be Predicted	R*	r†	Age (A)	Height (H)	Weight (W)	Surface Area (S)	Constant in the Equation	Coefficient of Variation
Male Subjects								
								per cent
Vital capacity, liters	0.866	0.855	−0.031(A)	+0.064(H)			−5.335	11
Residual volume, liters	0.647	0.636	+0.017(A)	+0.027(H)			−3.447	19
Total lung capacity, liters	0.774	0.769	−0.015(A)	+0.094(H)			−9.167	10
Functional residual capacity, liters		0.572		+0.081(H)		−1.792(S)	−7.11	17
Residual volume/total lung capacity × 100		0.779	+0.343(A)				+16.7	15
Functional residual capacity/total lung capacity × 100		0.558	+0.187(A)			−14.9(S)	+75.9	11
Maximal breathing capacity, (L/min.)	0.870	0.864	−1.873(A)			+78.2(S)	+72.7	14
Resting ventilation (L/min.)	0.675	0.518	+0.037(A)		+2.363(W)		+4.378	12
Female Subjects								
Vital capacity, liters	0.756	0.744	−0.018(A)	+0.052(H)			−4.36	13
Residual volume, liters	0.586	0.548	+0.009(A)	+0.032(H)			−3.90	24
Total lung capacity, liters	0.727	0.716	−0.008(A)	+0.079(H)			−7.49	11
Functional residual capacity, liters	0.636	0.610		+0.53(H)	−0.017(W)		−4.74	17
Residual volume/total lung capacity × 100	0.629	0.605	+0.265(A)				+21.7	18
Functional residual capacity/total lung capacity × 100	0.633	0.548	+0.146(A)		−0.428(W)		+ 75.9	13
Maximal breathing capacity (L/min.)	0.806	0.751	−0.965(A)			+67.18(S)	+26.3	15
Resting ventilation (L/min.)	0.562	0.548	−0.02(A)		+0.94(W)		+3.67	18

* Multiple correlation coefficient between measurement and the four body characteristics.

† Correlation coefficient between measurement and the one or two variables retained in the final formula.

if the present workers had used equipment with a lower resistance to airflow.

Values for resting ventilation, also exercise and standardized ventilation, and the dyspneic index calculated by the method of Hugh-Jones (14) are all shown in table 6. Resting, exercise, and standardized ventilation are all about a third higher than the figures reported by Hugh-Jones

TABLE 6

MEAN AND STANDARD DEVIATION OF VENTILATION VOLUMES MEASURED IN THE STEP TEST, MEAN FALL OF ARTERIAL SATURATION (RECORDED BY OXIMETRY) DURING THIS TEST, AND THE MEAN RISE IN ARTERIAL SATURATION DURING OXYGEN BREATHING,* AND THE MIXING INDEX†

	Male		Female	
	Mean	SD	Mean	SD
Resting ventilation, L/min	10.5	1.38	8.7	1.55
Exercise ventilation, L/min	31.4	4.83	32.3	4.73
Standardized ventilation, L/min	33.6	6.17	35.0	5.43
Arterial oxygen saturation				
Maximal fall per cent during standard exercise	3.4	1.39	2.9	2.07
Maximal rise per cent during oxygen breathing	6.5	1.27	6.6	1.25
Mixing index, liters of ventilation per liter of functional residual capacity	2.20	1.335	2.21	0.526

* Based on study of 41 male and 43 female subjects most of whom were included in the previous groups of 44 male, 50 female subjects.

† Based on the first group of 44 male and 50 female subjects.

at sea level for normal Welshmen (14), in spite of the fact that the step load for his subjects was 50 kg. m./min. higher than was completed by our subjects. An increase in ventilation both at rest and on exercise of approximately 30 per cent is in keeping with observations made in another laboratory in Johannesburg (29) and with the report of the Colorado workers at an altitude similar to that of Johannesburg (22).

The mean rise in percentage oxygen saturation of 6.5 per cent on breathing pure oxygen (table 6) implies a resting saturation of 93.5 per cent, i.e., 100−6.5 per cent.[7] This figure is somewhat lower than the value at sea level (28) and is again in agreement with other reports in the literature (22, 30) for altitudes of 6,000 feet above sea level.

Mixing indices are also shown in table 6. No direct comparison with other workers' results

[7] The rise in percentage saturation on oxygen breathing was the absolute rise noted in each individual case, e.g., if from a resting level of 97 per cent the saturation rose to 103 per cent, this was recorded as a rise of 6 per cent.

TABLE 7

CORRELATION OF MIXING INDICES WITH VARIOUS BODY CHARACTERISTICS

Correlation Coefficient of Mixing Index with	Male	Female
Age	+0.234	+0.352
Height	−0.456	−0.219
Weight	−0.007	+0.487
Surface area	−0.197	+0.299

was possible since this index has not been used elsewhere. The correlation coefficients of mixing indices with body characteristics, shown in table 7, suggest that this property of the lung is more closely related to the lung size (described by height in males and weight in females) than to its age. The lack of correlation between intrapulmonary mixing and age agrees with the work of Needham and associates (3), who also noted that mixing efficiency correlated with height in subjects aged eleven to eighteen years. Bates and Christie (31) and Briscoe (33), on the other hand, found that the mixing efficiency of a group of older subjects was less than that of younger subjects, the technique of these writers being similar to the one used in the present study. Similarly, Greifenstein (19) and his colleagues found that the intrapulmonary distribution of gases in older normal subjects, assessed by analysis of nitrogen in the expirate following a single breath of pure oxygen, was more uneven than in young normal people. It is possible that these differences between older people living in London (31, 33) and Philadelphia (19) and those living in Johannesburg and Aberdeen (3) may be related to climate and the prevalence of bronchitis in the larger cities. No other reason presents itself for the discrepancy between these observations.

The correlation of mixing efficiency and body size noted in this study was also found by Needham and co-workers in subjects less than nineteen years old, i.e., during the phase of most rapid growth, though not in older persons. It is of interest that Frank and associates (21) found a significant correlation between pulmonary compliance, on the one hand, and both height and vital capacity on the other hand; while Marshall showed that compliance related individually to functional residual capacity, residual volume, vital capacity, and total lung capacity (23). In other words, compliance appears

to be another property of the lung related to its size. It would not be surprising if the evenness of intrapulmonary mixing were in fact directly related to compliance. Unfortunately, the compliance of the subjects studied here was not measured so this relationship could not be examined.

Discussion

From this study certain conclusions can be drawn about lung function in subjects resident at median altitudes (5,760 feet above sea level). Some measurements appear to be no different from those reported from sea level laboratories. These are the subdivisions of lung volume, i.e., vital capacity, residual volume, and functional residual capacity, and their relation to lung volume, i.e., residual volume/total lung capacity × 100, functional residual capacity/total lung capacity × 100. The writers have in fact been struck by the similarity between results from different laboratories provided allowance is made for age and size differences in the subjects used by different workers to represent "normal." It follows, therefore, that one laboratory is justified in accepting standards for these measurements obtained in another laboratory.

There are certain values, however, which appear to be significantly different at median altitudes. These are the resting and exercise ventilation and maximal breathing capacity, all of which are higher than at sea level, and the arterial oxygen saturation, which is lower. The present findings are thus in agreement with those of other workers at median altitudes (22, 29). The mechanism causing differences in ventilation volumes and arterial saturation has been discussed in detail by others (22), and relates to the lower pO_2 in the air at altitudes.

With reference to the higher values for maximal breathing capacity, the present workers ascribe this difference also to the reduction in barometric pressure and thus differ from Anderson and co-workers (22). Cotes has concluded that a major factor accounting for the increase in maximal breathing capacity values at high altitude is likely to be the reduction in air density (32). Within the lung itself, reduced air density will reduce the work of breathing by reducing resistance to airflow at any given velocity. From Cotes' data and calculations, the effect of a reduction in lung gas density to about 0.8 of sea-level figures (as at an altitude of 5,760 feet above sea level) would cause maximal breathing capacity values to increase by approximately 6 per cent. In addition to its effect within the lung, the reduced air density at 5,760 feet above sea level will also affect the pressure-flow characteristics of the apparatus in which maximal breathing capacity is measured. It is not possible to estimate accurately how much maximal breathing capacity values may be increased due to these changes, and the apparatus used in this study has not been tested in a pressure tank to simulate sea-level conditions. Some idea of the maximal order of change likely to occur may, however, be obtained by application of formulas describing the relationship of flow to resistance in smooth pipes. In the case of laminar flow at any fixed velocity, the pressure (P) across the instrument would fall by a factor P × 0.967 in changing from 0 to 6,000 feet. The equivalent change for turbulent flow would be P × 0.775 × 0.994. It is probably justifiable to assume that the flows encountered during a maximal breathing capacity test in a Benedict-Roth spirometer are almost entirely turbulent. Thus, in an instrument in which the pressure required to produce a flow of 2 liters per second is about 2.2 cm. of water at 5,760 feet; at sea level about 2.8 cm. of water pressure would be required to achieve the same flow. It has been shown elsewhere (13) that changes in the pressure flow characteristics of the measuring system of this order can be expected to reduce maximal breathing capacity values by about 9 per cent. In other words, the effects of the reduced air density on maximal breathing capacity are likely to be an approximate increase of 6 per cent, due to reduction in the work of breathing, and 9 per cent, due to changes in the pressure flow characteristics of the recording system, a total of 15 per cent. It may be seen in table 1 that formulas based on the present study over-predict maximal breathing capacity values for subjects studied at sea level by amounts varying from 3 to 25 per cent. These percentages might well be closer to 15 per cent if all workers had used apparatus in which the resistance characteristics were similar to those of the one used here. Nevertheless, it seems reasonable to conclude that the higher values for maximal breathing capacity recorded in this study are such as might be expected to result from reduced air density at altitude. Whether increased power of the chest, due to perpetually higher levels of ventilation, is also a factor in producing the

higher maximal breathing capacity values in subjects resident at altitude is as yet unproved.

Summary

Values for the subdivisions of lung volume of subjects acclimatized at 5,760 feet above sea level do not appear to differ significantly from sea-level values if account is taken of the differences in body size. On the contrary, the similarity of values reported from different laboratories is striking. In contrast, maximal breathing capacity and resting and exercise ventilation are significantly higher, and the resting arterial oxygen saturation is significantly lower at median altitudes. This is in keeping with the findings of other workers. Formulas have been derived for predicting various subdivisions of lung volume from age and body characteristics, and it is shown that it is justifiable to apply these formulas both at sea level and at altitudes at least up to 5,760 feet.

Sumario

Pruebas de la Función Respiratoria: Valores Normales a Alturas Medianas y la Predicción de Resultados Normales

Los valores para las subdivisiones del volumen pulmonar de los sujetos aclimatados a unos 1,750 metros sobre el nivel del mar no discrepan mayor cosa de los valores al nivel del mar si se toman en cuenta las diferencias en el tamaño del cuerpo. Por el contrario, es notable la semejanza de los valores descritos de distintos laboratorios. En contraposición, la capacidad respiratoria máxima y la ventilación en descanso y durante el ejercicio son decididamente más altas, y la saturación arterial de oxígeno en descanso es decididamente más baja a alturas medianas. Esto armoniza con los hallazgos de otros investigadores. Se han derivado fórmulas para predecir varias subdivisiones del volumen pulmonar por la edad y las características físicas, y se demuestra que está justificado aplicar estas fórmulas tanto al nivel del mar como hasta alturas a lo menos de unos 1,755 metros.

Resume

Tests de la fonction respiratoire: valeur normale aux altitudes moyennes et prévisions des résultats normaux

Les valeurs pour les subdivisions du volume pulmonaire des sujets acclimatés à une altitude d'environ 1.750 mètres au-dessus du niveau de la mer ne paraissent pas différer de façon notable des valeurs obtenues au niveau de la mer, si l'on tient compte des différences de taille. Au contraire, la similarité des valeurs rapportées par différents laboratoires est saisissante. En contraste, la capacité respiratoire maxima et la ventilation durant l'exercice et au repos sont significativement plus élevées, tandis que la saturation en oxygène artériel au repos est significativement plus basse aux altitudes moyennes. Ceci corrobore les résultats obtenus par d'autres chercheurs. Des formules dérivées de l'âge et des caractéristiques corporelles pour estimer à priori les diverses subdivisions du volume pulmonaire ont été élaborées. Il a été démontré qu'au niveau de la mer et aux altitudes allant jusqu'à 1.750 mètres l'application de ces formules est justifiée.

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PULMONARY FUNCTION CHANGES IN BILATERAL RESECTION FOR PULMONARY TUBERCULOSIS[1, 2]

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INTRODUCTION

Bilateral pulmonary resection has become an accepted adjunctive form of treatment for pulmonary tuberculosis in recent years. It is undertaken frequently as an initially planned procedure in selected cases when definite indications exist and respiratory function is found to be adequate. In other cases it is considered but not decided upon until the patient fully recovers from the first resection and pulmonary function is again carefully assessed. It is in connection with this latter group that it often becomes vitally important to obtain accurate information concerning the amount of respiratory reserve remaining. This applies also in certain complicated cases with already significantly impaired function, in which resection on one side only might reduce respiratory reserve to a critical level.

In addition to presurgical clinical assessment of the functional status of the individual, much help can often be derived from certain ventilatory tests when performed carefully and intelligently, using acceptable apparatus. In borderline cases, this testing should include bronchospirometry as well as the more standard methods of determining the total function.

Changes in pulmonary function following unilateral resection for tuberculosis have been studied and recorded by several investigators (1–5). It has been demonstrated conclusively that resection of diseased tuberculous segments, including lobectomy, often produces rather small, if any, reduction in pulmonary function in uncomplicated cases.

Although bilateral resection for pulmonary tuberculosis has been employed fairly extensively since 1953, there have been very few published studies relating to pulmonary function changes. Kraan (6) reported in detail the changes observed in six patients who had ventilatory tests performed before and after the first resection and following the second operation. He noted that the changes in function were very small after bilateral segmentectomies: the vital capacity was reduced only by insignificant amounts and the maximal respiratory minute volume either increased or diminished only very slightly. In a later study, Falk and Jensen (7) reported satisfactory pulmonary function in most of their series of sixty patients following bilateral resection. Several of their patients had lobectomy on one side. Unfortunately, a detailed functional test study was not performed and impressions were based largely on clinical evaluation.

The present study was undertaken primarily to determine as accurately as possible the degree of change in pulmonary function following bilateral resection of varying extent.

PLAN OF INVESTIGATION

Clinical Material

Twenty-nine patients who had bilateral pulmonary resection of tuberculous lesions between June, 1955, and February, 1958, were selected for study. This number was part of a group of more than 60 who had had bilateral resections performed at the Nova Scotia Sanatorium since 1953, review of which is to be published in the near future. Only the 29 selected for this study had complete and identical ventilatory testing performed. All of these subjects had received previous bed rest and antimicrobial therapy for varying periods. Six had relaxation procedures performed at some time prior to surgery, 2 had pneumothorax on one side a few years earlier, and 4 had pneumoperitoneum for at least a year. In all cases these procedures had been abandoned by the time of their first resection. None had a previous thoracoplasty or phrenic nerve operation.

Sixteen of the subjects were males, and the ages ranged from seventeen to forty-nine years with the majority in the twenty to thirty-nine age group (table 1).

Nineteen of the patients had tuberculous disease that was classified as moderately advanced at the time of admission to the hospital (table 1). The

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disease to be resected was located in the upper lobes, bilaterally, in all 29 patients. The indications for surgical intervention were residual fibrocaseous nodular disease, persisting cavitation, and bronchiectasis, or a combination of these types of disease. Disease known to be unstable and the presence of tubercle bacilli in the sputum influenced the decision regarding surgery in many instances. The operative procedures were performed by the same team of surgeons in the majority of patients, and technique varied very little. An attempt was always made to preserve as much normal lung tissue as possible while resecting the most significant lesions. Rapid re-expansion of the remaining lung was sought, and complications were very few in the group studied. None of these patients required or received space-filling thoracoplasty subsequent to resection of lung tissue. Postoperative breathing exercises were employed routinely with close instruction to prevent if possible the occurrence of atelectasis or a fixed diaphragm and thoracic wall. These exercises were continued under supervision for several months after surgery.

The extent of the operations varied considerably. A bilateral single-wedge resection was the combination in 10 patients. In 8 patients, one lobe and one wedge were resected; in 4 patients, one segment and one wedge were resected. A lobectomy with two segmental resections was performed in 2 patients, and a lobectomy with one segmental resection in 2 others. The remaining 3 patients had resection of: two segments and one wedge; one segment and two wedges; and three wedges. The most extensive resections were a lobectomy on one side and two segments on the other performed on 2 patients. In all, 12 patients had lobectomies on one side. An "in-between" group of 7 patients had one or two wedges resected on one side and either two wedges, two segments, one segment, or a segment and wedge on the other. The interval between operations varied from ten weeks to ten months with an average of five months.

TABLE 1

PULMONARY FUNCTION STUDY—BILATERAL RESECTIONS

Age and Sex Distribution and Extent of Disease Related to Age Groups

Age Group	Sex		Extent of Disease			Totals
	Male	Female	Minimal	Moderately Advanced	Far Advanced	
10–19	—	4	2	2		4
20–29	8	6		10	4	14
30–39	3	2		4	1	5
40–49	5	1		3	3	6
	16	13	2	19	8	29

Youngest—17 years, 8 months at time of second operation.

Oldest—49 years, 10 months at time of second operation.

METHODS

The patients were studied in respect to pre- and postoperative condition and pulmonary function assessment. A routine was established which consisted of: noting any important past or present conditions such as asthma, bronchitis, pleurisy with effusion, previous thoracic operative procedures, and complications; noting the presence or absence of such symptoms as dyspnea on mild or moderate exertion, wheezing, and chest pain; careful fluoroscopy, noting any evidence of pulmonary emphysema, any abnormality of the mediastinum and, in particular, the degree of costal and diaphragmatic excursion on deep respiration (occasionally this procedure was supplemented by inspiratory and expiratory roentgenograms, and in all cases the information gained was related to physical examination of the chest and standard postero-anterior films, planigrams, and bronchograms); ventilatory testing, including the determination of minute resting ventilation, expiratory reserve volume, vital capacity, and maximal breathing capacity.

This assessment was performed prior to the first operation and repeated following the first and second operations. In all but a few instances the tests were performed less than three months before the first operation and between three to six months after each of the two resections. Each pulmonary function assessment, including the performance of ventilatory tests, was carried out in essentially the same manner by one of two physicians with the assistance of one of three technicians. A Collins 9-liter direct recording respirometer was used for all testing, and corrected calculations were made with the aid of commonly employed standardized formulas.[4] In determining the maximal breathing capacity, a 12-second interval was used in order to avoid placing unduly heavy strain on the patient and also to facilitate calculations. In all testing an attempt was made to induce the maximal effort from the subject at all times. Testing was done with the patient sitting in a comfortable position after the procedure was thoroughly explained. At least three tests of vital capacity and maximal breathing capacity were performed on each occasion with the best result of each recorded. For each subject the calculated normal or predicted result was related to the actual value obtained.

RESULTS

The over-all changes in vital capacity and maximal breathing capacity for the entire group of patients studied were computed from the actual

[4] Baldwin, Cournand, and Richards.

TABLE 2

PULMONARY FUNCTION STUDY—BILATERAL RESECTIONS

Ventilatory Changes in Relation to Extent of Resection

Group	Number of Cases	Ventilatory Tests: Vital Capacity (ML) (Range, Median, and Average) — Before Operation: Actual	Before Operation: Per Cent of Predicted	Number of Increases	Number of Decreases	Total Change Between First and Last Tests: Actual	Total Change Between First and Last Tests: Per Cent of Actual	Total Change Between First and Last Tests: Per Cent of Predicted	Maximal Breathing Capacity (Liters per Minute) (Range, Median, and Average) — Before Operation: Actual	Before Operation: Per Cent of Predicted	Number of Increases	Number of Decreases	Total Change Between First and Last Tests: Actual	Total Change Between First and Last Tests: Per Cent of Actual	Total Change Between First and Last Tests: Per Cent of Predicted
A*	10	Range (2,101 to 5,689)	(67 to 120)	1	9	(+99 to −1,039)	(+4 to −21)	(+4 to −20)	Range (79 to 148)	(54 to 122)	6	4	(+27 to −16)	(+34 to −12)	(+37 to −8)
		Median 4,099	104			−653	−16	−15	Median 114	94			+9	+4	+7
		Average 3,987	100			−570	−13	−12	Average 111	92			+6	+5	+8
B†	7	Range (1,833 to 4,063)	(63 to 102)		7	(−288 to −1,050)	(−7 to −31)	(−7 to −30)	Range (56 to 135)	(71 to 110)	4	3	(+23 to −22)	(+34 to −17)	(+34 to −19)
		Median 3,253	86			−388	−14	−13	Median 99	85			+2	+1	+1
		Average 2,994	84			−485	−16	−16	Average 92	89			+3	+7	+8
C‡	12	Range (2,325 to 4,124)	(67 to 100)		12	(−10 to −1,189)	(0 to −38)	(0 to −38)	Range (66 to 132)	(71 to 136)	3	9	(+40 to −38)	(+61 to −29)	(+74 to −29)
		Median 2,917	85			−573	−21	−21	Median 92	91			−16	−17	−13
		Average 3,129	85			−637	−20	−20	Average 100	91			−10	−8	−5

* Patients who had bilateral single wedge resections

† Patients who had greater total resection than *Group A* but less than *Group C*

‡ Patients who had lobectomy on one side and lesser resection on the other

and the predicted results. It was of interest to note that the preoperative figures expressed in terms of per cent of predicted values did not show any close correlation to the extent of disease present. A minority of those patients who required the more extensive resections had any significant reduction in the initial tests. A few patients had an increase in vital capacity following the first operation, with a reduction following the second. About the same number had a decrease after the first operation, but had an increase after the second. Only one patient had a total or net increase when the tests before the first operation were compared with those following the second procedure.

The median net change in vital capacity for all of the patients was a reduction of 18 per cent of actual and 17 per cent of predicted values. Several patients had a net increase in maximal breathing capacity between the first and last tests. However, for the group there was a median decrease of 5 per cent of actual and 3 per cent of predicted values.

In table 2 the patients are divided into three groups in order to compare the results in relation to the extent of resection. *Group A* includes 10 patients who had bilateral single-wedge resections: there was a median decrease of 16 per cent in actual vital capacity and an increase of 4 per cent in actual maximal breathing capacity. A net increase in maximal breathing capacity figures occurred in 6 patients of this group.

Those patients having at least a lobectomy on one side with a variable lesser resection on the other are included in *Group C*. In every instance the lobe resected was the upper. This group of 12 patients had a median net decrease in vital capacity of 21 per cent of actual values. The maximal breathing capacity diminished by 17 per cent of actual and 13 per cent of the predicted value. A few of these patients who had a total of four or five segments resected had fairly marked increases in maximal breathing capacity.

Group B includes the "in-between" cases which had resections totaling more than two wedges but less than a lobectomy on either side. In this small group the vital capacity decreased by 14 per cent and the maximal breathing capacity increased by 1 per cent of the actual values.

The tests were repeated on 7 patients a second time after their second operation. The interval between the second operation and the first of these final tests ranged from three to seven months with an average of four, while the additional interval before the second test was performed varied from two to five months with an average of three. During this interval, 3 patients had an increase in vital capacity with a median of 5 per cent of actual. One patient had no change, while the remaining 3 had a median decrease of 14 per cent of actual. This included the single patient in the entire group who had a net increase between tests before the first operation and the last tests following the second resection. Four of the group of 7 had an increase in maximal breathing capacity with a median of 5 per cent of actual. The median decrease for the remaining 3 was 8 per cent of actual, and this included 2 patients who had shown a net increase in values between the tests before the first, and the final tests after the second, operation.

An attempt was made to relate individual results of vital capacity and maximal breathing capacity before and after resections to such factors as: history of significant preoperative chest condition, including previous relaxation procedures; symptoms of dyspnea and wheezing; postoperative complications; and fluoroscopic evidence of impaired costal and diaphragmatic movement on deep respiration. No correlation for the group could be established. Although occasionally some factor seemed to influence the results, much more frequently these factors apparently had little or no relationship to the changes in vital capacity or maximal breathing capacity. For instance, 2 of the patients who had marked restriction of diaphragmatic excursion bilaterally following operations were in the group of those who had over-all increases in maximal breathing capacity, and one was the single subject to have also an over-all increase in vital capacity. Again, it was difficult to make comparisons in relation to these factors because of the variance in extent of resections.

Discussion

As determined by the ventilatory tests employed, the reduction in pulmonary function in this series of patients was very small. The vital capacity decreased appreciably more in the major resection group (those having lobectomy) than in the minor, less extensive, resection groups. This finding differs from that of Taylor and associates (4) who concluded that the vital capacity loss was remarkably similar in all of their one- to

† Patients who had greater total resection than *Group A* but less than *Group C*

‡ Patients who had lobectomy on one side and lesser resection on the other

seven-segment unilateral resection groups. Only in their pneumonectomy group of five cases did they find a significantly greater loss than in lesser resections. They, as well as Pecora (5), attribute the similarity in the degree of reduction of vital capacity in the less-than-seven-segment resections to a thoracotomy or restrictive effect, and the result of confining resection to diseased segments, preserving normally ventilating lung.

The reduction in vital capacity of 18 per cent for the entire group compares favorably with the approximate loss of 20 per cent determined by Taylor and associates (4) in a large group of unilateral resections. This is of special interest when it is considered that in bilateral resection there is a double thoracotomy effect.

The results of maximal breathing capacity tests indicate a very minor change in function for the less extensive resection groups. Several patients who had an actual increase may or may not have experienced improved function as a result of their surgery. Many factors influence the maximal breathing capacity, including learning ability to perform tests, general condition, and motivation, in addition to bronchopulmonary and chest wall conditions. Any improvement of function which may have occurred in the small resection groups can almost certainly be attributed to such factors as improved general condition and more efficient mechanics of breathing. The latter seemed to be of importance in a few patients who benefited considerably from breathing exercises in the post-resection period. Nevertheless, it cannot be discounted that the level of motivation constitutes an ever present and potentially considerable source of error in the results. That the extent of resection influences the pulmonary function as reflected in the maximal breathing capacity is very clear when the results in the major and minor resection groups are compared. In the minor groups as a whole there was no reduction in the tests, whereas the group which had major resection (lobectomy on one side) experienced a median decrease of 17 per cent of actual or 13 per cent of predicted values (*see* table 2). It is quite probable that this reduction in most instances was due to removal of ventilating lung tissue. Overinflation of the remaining lung may occasionally be a contributing factor. The increases noted in 3 patients may represent an actual improvement of function, possibly produced by removal of grossly diseased bronchopulmonary segments which were interfering with ventilation and respiration. The possibility of a false improvement in function tests explained on the basis of learning ability, motivation, and fitness must also be considered.

Summary

The results of ventilatory tests and clinical assessment in this study indicate that bilateral resection of reasonable extent affects the pulmonary function to a relatively slight degree.

Reduction in vital capacity was fairly constant for the minor resection groups and amounted for the median to 15 per cent of actual and 14 per cent of predicted values. In the major group of patients, those who had lobectomies on one side, the comparable figures were 21 per cent. For the entire group the median reduction was 18 per cent.

Maximal breathing capacity changes correlated to the extent of resection better than did vital capacity, the reduction being in the vicinity of 17 per cent of actual and 13 per cent of predicted values in the major resection group. This contrasts with the results in the minor resection groups which indicated no reduction. The median reduction for the entire group was 5 per cent.

The probable major cause for reduction of vital capacity is considered to be a thoracotomy effect which is of a restrictive nature. In the more extensive resections, pulmonary factors undoubtedly influence the results. On the contrary, maximal breathing capacity is apparently unaffected by the thoracotomy factor when tests are performed several weeks after resection.

The maximal breathing capacity test, although valuable, is not infallible because of the potential sources of error connected with its performance. A correlation between other factors, including age, pre-existing conditions, and type of disease, with the group results could not be demonstrated.

Sumario

Alteraciones de la Función Pulmonar en la Resección Bilateral por Tuberculosis Pulmonar

Los resultados de las pruebas ventiladoras y de la justipreciación clínica en este estudio indican que la resección bilateral de una magnitud razonable afecta relativamente poco la función pulmonar.

La reducción de la capacidad vital fué bastante constante para los grupos de resección menor,

promediando 15 por ciento de la cifra real y 14 por ciento de la predicha. En el grupo mayor de enfermos en que se ejecutó una lobectomía unilateral, las cifras comparables fueron de 21 por ciento. Para todo el grupo, la reducción media fué de 18 por ciento.

Las alteraciones de la capacidad respiratoria máxima se correlacionaron mejor que la capacidad vital con el tamaño de la resección, quedando la reducción en la proximidad de 17 por ciento de la cifra real y 13 por ciento de la predicha en el grupo de resección mayor. Esto choca con los resultados obtenidos en los grupos de resección menor, que no indicaron reducción alguna. La reducción media para todo el grupo fué de 5 por ciento.

La principal causa probable de la reducción de la capacidad vital parece ser un efecto toracotómico, que es de naturaleza restrictiva. En las resecciones más extensas, los factores pulmonares afectan indudablemente el resultado. En cambio, la capacidad respiratoria máxima no se ve aparentemente afectada por el factor toracotómico, cuando se ejecutan pruebas varias semanas después de la resección.

La prueba de la capacidad respiratoria máxima, aunque valiosa, no es infalible debido a las causas potenciales de error que intervienen en su ejecución. No pudo descubrirse correlación alguna entre otros factores, comprendiendo edad, condiciones preexistentes y forma de la enfermedad, con los resultados obtenidos en los distintos grupos.

Resume

Modifications de la fonction pulmonaire dans la résection bilatérale pour tuberculeuse pulmonaire

Les résultats des tests de ventilation et l'évaluation clinique effectuée dans cette étude indiquent que la résection bilatérale d'étendue raisonnable n'affecte la fonction pulmonaire qu'à un degré relativement faible. La réduction de la capacité vitale était assez constante dans le groupe ayant subi les résections les plus réduites, étant en fait de 15% en moyenne, par rapport aux 14% prévus. Dans le groupe soumis aux résections majeures, lobectomie unilatérale, le pourcentage comparable était de 21%. Pour le groupe entier la réduction moyenne était de 18%.

Les modifications de la capacité respiratoire maxima montraient une meilleure corrélation avec l'étendue des résections que la capacité vitale, la réduction étant d'environ 17% en fait contre celle prévue de 13% pour le groupe des résections majeures. Ceci est en contraste avec les résultats obtenus dans les groupes de résections mineures, ceux-ci n'indiquant aucune réduction. La réduction moyenne pour le groupe tout entier était de 5%.

La cause majeure probable de la réduction de la capacité vitale est considérée comme étant une thoracotomie à effet restrictif. Dans les résections plus extensives les facteurs pulmonaires influencent indiscutablement les résultats. Au contraire, les tests effectués plusieurs jours après une résection indiquent que la capacité respiratoire maxima ne paraît pas affectée par le facteur thoracotomie.

Le test de capacité respiratoire, tout en ayant de la valeur, n'est pas infaillible en raison du potentiel d'erreurs qu'il comporte. Une corrélation entre d'autres facteurs, y compris l'âge, les conditions pré-existantes et le type de la maladie et les résultats obtenus pour le groupe n'a pu être établie.

Acknowledgment

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A PRACTICAL ROUTINE FOR THE PRESURGICAL EVALUATION OF LUNG FUNCTION IN PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

Tests of pulmonary function in both medical and surgical lung disease have become increasingly popular in recent years. These have aided the chest physician in diagnosis, therapy, and a more accurate understanding of the physiology of lung disease. The chest surgeon is more interested in the ability of his patient to withstand an anesthetic, a major thoracic intervention, the immediate postoperative period, and the long-term effect of his operation with respect to disability.

Almost coincidental with the advent of clinical lung-function tests has been the change in the chest surgery of tuberculosis. The tendency has been to discontinue such procedures as pneumothorax, pneumoperitoneum, phrenicotomy, thoracoplasty, and pneumonectomy and to use instead resection of lobes, segments, and wedges.

There is considerable literature available on the physiopathology of tuberculosis in general and the loss of function after the surgical procedures listed above, but there is some vagueness in the literature when an attempt is made to find a brief and efficient routine for the presurgical evaluation of lung function and the criteria that should be used in making the decision for or against surgery. Perhaps this vagueness is necessary to some extent, since there must be a wide variation in the standards of each laboratory, anesthesiologist, surgeon, and nursing unit. The criteria used in making a surgical decision in one hospital would differ from those in another. Nevertheless, it is believed that a review and analysis of cases studied over a two-year period in the pulmonary function laboratory of this hospital might be of use in clarifying some of the above factors.

MATERIALS AND METHODS

All patients with pulmonary tuberculosis who were formally proposed for chest surgery during the two years 1955 and 1956 were given vital capacity and maximal breathing capacity tests. Residual volume, arterial oxygen saturation, arterial carbon dioxide content, and bronchospirometry were measured on the following three groups of patients: those whose maximal breathing capacity was less than 80 per cent of the predicted value, those whose maximal breathing capacity was greater than 80 per cent of the predicted value but on whom bilateral surgery was planned (at a six-month interval), and those on whom pneumonectomy was planned.

Toward the end of the study, some of these tests were not believed to be necessary and so were not performed. With regard to maximal breathing capacity, more attention was paid to the percentage of the predicted value than to the absolute figures, as this test varies with age, body surface area, and sex, and so produced widely varying absolute figures.

Vital capacity tests were performed on a 13.5-liter Benedict-Roth spirometer. Maximal breathing capacity was determined by collecting and recording in a Tissot spirometer the gas expired from a Hans Rudolph valve over a fifteen-second period. Predicted values for both vital capacity and maximal breathing capacity were obtained from the formulas of Baldwin and associates (1). Residual volume was measured by a modification of the open-circuit method of Darling, Cournand, and Richards (2). The index of intrapulmonary mixing was determined by Van Slyke analysis of the alveolar sample of gas taken at the end of this test. Predicted residual volume was obtained from the difference between the predicted total lung capacity and predicted vital capacity, and the former was obtained from the figures of Aslett and co-workers (3). Bronchospirometry was carried out with a Carlen's catheter inserted under direct vision; and minute volume, vital capacity, and oxygen absorption were recorded on twin McKesson spirometers.

Arterial blood was obtained from the brachial artery in heparinized syringes under anaerobic conditions. These were analyzed for carbon dioxide content, oxygen content, and oxygen capacity, the oxygen saturation being considered as the ratio of the latter two by the method of Peters and Van Slyke (4).

Postoperative complications were obtained from a punch-card system recorded by the surgeon. Complications were defined as follows: atelectasis (more than one lobe), delayed re-expansion (more than one week), bronchopleural fistula, wound

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infection, spread of tuberculosis (infiltration persisting more than one week), subcutaneous emphysema, postoperative hemorrhage (more than 300 ml. in twenty-four hours), paradoxical respiration, empyema, and respiratory insufficiency. This latter term was defined as unusual shortness of breath in the postoperative period of three weeks.

Ninety-two of the 322 patients who were operated upon performed a vital capacity and maximal breathing capacity test six months or more after surgery. These were picked at random from the follow-up surgical clinic. The tests were done on the same apparatus and, when possible, by the same technician. The mean reduction of vital capacity and maximal breathing capacity was expressed as a percentage of the preoperative value. In addition, the percentage loss of function was related to the amount of lung tissue removed. Since all segments do not have the same volume, the following unitage system was used:

> Segment 3 of each upper lobe and segment 6 of each lower lobe were considered as 2 units; the remainder, including wedges, as one unit. None of the patients studied postoperatively had basalar segments removed. Tailoring thoracoplasty was performed on 2 patients in *Group I*, 2 in *Group II*, and one in *Group III*. Each patient was questioned as to the appearance or increase of shortness of breath. Since the number of pneumonectomies was so small, postoperative studies were not done on this group.

Results

During the two years in question, 417 patients were studied. These fell into four groups according to the reason for and extent of pulmonary investigation.

Group I: The maximal breathing capacity in *Group I* was greater than 80 per cent of the predicted value for those for whom unilateral surgery was planned. As may be seen in table 1, 308 of the 417 patients (74 per cent) formed this group, but only 254 (82 per cent) were operated upon despite adequate function. The remaining 54 (18 per cent) were refused surgery for various reasons not related to lung function. One hundred and eleven of the 254 (44 per cent) had postoperative complications; 25 patients (10 per cent) had multiple complications. None of this group had respiratory insufficiency in the immediate postoperative period. There was one death in a patient who had been treated years before with oleothorax. He developed a bronchopleural fistula and, after decortication, died of empyema.

Group II: The maximal breathing capacity in *Group II* was greater than 80 per cent of the predicted value for those for whom bilateral surgery was planned. Forty-four of the 417 patients (11 per cent) fell into this group. Complete studies were made on all but 3 of the patients.

Lung function tests are not recorded here in detail. However, the maximal breathing capacity was greater than 80 per cent of the predicted value in all cases. The residual volume was less than 125 per cent of the predicted value in 20 patients, and was moderately increased in the remaining 21. In 7 of the latter, however, the total lung capacity was also increased, so that 27 of the 41 patients had a residual volume/total lung capacity of less than 30 per cent. The total lung capacity in the remaining 14 was only slightly increased. In only 4 was the index of intrapulmonary mixing more than 2.5 per cent, suggesting a minor abnormality of alveolar gas distribution. The arterial oxygen saturation was

TABLE 1

Percentage of Patients Accepted and Refused in 417 Cases Proposed for Surgery
Complications in 322 Operations

Group	Age		Patients		Accepted for Surgery		Refused for Lung Dysfunction		Complications					
	Mean	Range	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number of Patients	Per Cent	Multiple Complications		Respiratory Insufficiency	Deaths
											Number	Per Cent		
	years													
I	36.7	14–74	308	74	254	82	0	0	111	44	25	10	0	1
II	34	22–52	44	11	38	86	0	0	24	63	7	18	1	2
III	43	22–66	57	14	23	40	18	31	19	83	12	26	0	0
IV	42	32–65	8	2	7	88	0	0	3	43	3	43	1	2

less than 92 per cent in only one patient of the 41 on whom the measurement was performed, and in none was carbon dioxide retention observed. Bronchospirometry showed widely varying results, since the extent and distribution of the lesions varied widely.

Of the 44 patients in this group (*see* table 1), only 38 (86 per cent) were operated upon, the remainder being refused surgery for reasons not related to their lung function. Twenty-four patients (63 per cent) had complications and 7 (18 per cent) had multiple complications. One patient had questionable respiratory insufficiency postoperatively. There were 2 deaths, one due to rupture of the esophagus and the other from a bronchopleural fistula.

Group III: The maximal breathing capacity in *Group III* was less than 80 per cent of the predicted value. The pulmonary function tests in this group are recorded in table 2. Incomplete studies included patients who were refused surgery prior to completion of the tests and those on whom complete tests were not found to be necessary as a result of experience gained in the early part of the study.

The residual volume and residual volume/total lung capacity were high in most of the cases, as was the index of intrapulmonary mixing. An arterial oxygen saturation of less than 92 per cent occurred in 11 of the 37 patients on whom the test was performed. Only one of the 37 patients had a high carbon dioxide content. The bronchospirometry results are based on minute volume.

It may be seen in table 1 that this group was composed of 57 of the 417 patients (14 per cent). Only 23 (40 per cent) were accepted for surgery, 18 (31 per cent) being refused on the basis of poor pulmonary function and 16 (29 per cent) for reasons not directly due to pulmonary dysfunction. Of the 23 operated upon, 19 (83 per cent) had complications and 12 (26 per cent) had multiple complications. There was no respiratory insufficiency in the postoperative period and no deaths occurred.

Group IV: Proposed pneumonectomies: There were 8 patients (2 per cent) of the 147 in *Group IV*. Their maximal breathing capacities ranged from 45 to 64 per cent of the predicted value with the exception of one whose maximal breathing capacity was 93 per cent. The residual volume was more than 125 per cent in all but one case, but the residual volume/total lung capacity was more than 30 per cent in all cases. The index of intrapulmonary mixing was greater than 2.5 per cent in all but one. One patient had an arterial oxygen saturation of 91.4 per cent, but the arterial oxygen saturation in all others was more than 92 per cent. In none was there carbon dioxide retention.

Of the 8 patients in this group, 7 (88 per cent) were accepted for surgery, the remaining one being refused for reasons not related to his pulmonary function. Three (43 per cent) of the 7 patients had postoperative complications, all of them multiple. One patient had questionable postoperative respiratory insufficiency. There were 2 deaths, one from rupture of the pulmonary artery during the operation and one from bronchopleural fistula and pulmonary edema.

The postoperative changes in vital capacity and maximal breathing capacity are shown by group in table 3. The number of units of lung tissue removed was slightly greater in *Group III* (3.9 units) than in *Groups I* (3.3 units) and *II* (3 units). The reduction in vital capacity was 6, 15, and 11 per cent in *Groups I*, *II*, and *III*.

The reduction in maximal breathing capacity did not vary greatly in reference to absolute figures, there being a reduction of 9, 14, and 10 liters per minute in *Groups I*, *II*, and *III*, respectively; but the percentage loss was greater in *Group III* since the preoperative mean level was 75 liters per minute, as compared to 124 and 129 liters per minute in *Groups I* and *II*. The appearance of, or increase in, dyspnea six months postoperatively is also shown in table 3. Twelve patients in *Group I*, one in *Group II*, and 2 in *Group III* complained of this symptom.

The changes in vital capacity and maximal breathing capacity are shown by unit in table 4. The number of cases studied in each unit group was small, especially in those who had six or seven units removed. For this reason the latter will not be considered.

Postoperatively there was a 6 per cent reduction in vital capacity when one, two, and five units were removed, and a 9 per cent reduction when three and four units were lost at operation.

When one and two units were removed, the mean postoperative reduction in maximal breathing capacity was 3 and 0.9 per cent respectively, but when three to five units were removed an 8 to 12 per cent reduction in maximal breathing capacity occurred.

TABLE 2

LUNG FUNCTION ON 57 PATIENTS OF GROUP III

Number of Patients	Vital Capacity		Maximal Breathing Capacity		Residual Volume		RV/TLC	IIM*	Arterial Oxygen Saturation	Arterial Carbon Dioxide Content	Broncho-spirometry	Surgery
	Observed Values	Per Cent	Observed Values	Per Cent	Observed Values	Per Cent	Per Cent	Per Cent	Per Cent	Vol. Per Cent	R/L	
1	2,979	73	49	42	1,618	159	35	5.76	94.2	46.76	39/61	R.P.
2	2,491	60	48	53	1,957	155	42	2.96	93.7	51.62	—	R.O.
3	2,401	79	52	67	1,497	161	37	1.74	94.4	43.30	—	A.
4	3,188	73	83	60	1,278	118	27	8.48	90.7	44.73	55/45	A.
5	3,512	87	56	54	1,787	154	33	7.08	93.5	49.78	55/45	A.
6	2,514	59	56	48	1,546	119	41	2.02	91.0	50.97	—	R.P.
7	2,038	52	44	46	1,712	99	46	4.85	94.8	47.13	45/55	R.P.
8	3,113	72	72	65	2,245	151	41	6.03	—	—	39/61	A.
9	3,592	89	90	76	2,502	203	41	1.49	94.9	47.15	48/52	A.
10	3,323	78	67	57	2,132	163	39	3.38	—	—	34/66	A.
11	2,863	81	70	76	2,964	186	51	1.96	86.1	43.43	—	R.O.
12	3,187	75	84	77	1,807	139	36	2.77	95.6	52.06	52/48	A.
13	2,114	46	71	55	947	83	31	2.07	93.2	46.93	77/73	R.O.
14	1,057	35	26	33	1,513	199	57	2.18	93.8	47.24	100/0	R.O.
15	2,134	65	57	68	1,099	109	34	2.39	96	45.38	22/73	R.O.
16	3,682	88	55	50	2,635	178	41	6.10	90.9	50.32	—	A.
17	3,246	75	96	69	1,807	168	38	4.29	93.3	49.28	86/14	A.
18	3,615	88	69	56	1,120	87	25	2.91	90	46.93	55/45	A.
19	3,640	107	60	70	1,586	153	32	2.09	89	48.67	50/50	A.
20	3,869	82	85	63	—	—	—	—	95.1	50.54	52/48	A.
21	2,650	57	55	40	1,730	167	39	4.48	89.6	42.54	54/46	R.P.
22	2,265	70	51	55	1,442	178	38	2.47	94.5	45.22	62/38	A.
23	2,312	77	40	48	1,688	196	41	5.22	98	52.06	68/32	R.P.
24	2,093	46	69	54	—	—	—	—	92.6	52.23	91/9	R.P.
25	3,575	84	76	54	2,267	213	39	3.26	94.5	47.42	81/19	A.
26	1,806	44	37	37	—	—	—	—	96.2	50.20	82/18	R.P.
27	3,655	85	63	57	2,487	232	39	5.09	93.2	53.45	—	A.
28	2,582	56	88	70	3,096	221	54	2.22	88.2	47.57	34/66	A.
29	3,347	76	85	66	1,400	109	29	2.86	93.8	45.27	73/27	R.O.
30	2,135	50	68	54	—	—	—	—	—	—	48/52	A.
31	4,939	108	87	66	2,147	154	31	8.25	93	45.02	60/40	A.
32	2,829	66	77	65	1,025	78	26	2.15	86.7	46.81	—	A.
33	1,334	47	46	53	1,161	133	46	4.51	94.8	43.66	89/11	R.P.
34	2,020	62	63	64	1,739	206	48	2.94	94.2	46.12	88/12	A.
35	2,356	83	44	60	2,416	280	51	4.17	97.8	42.76	81/19	A.
36	3,157	72	77	70	1,742	130	35	2.63	94.7	47.9	45/55	A.
37	2,759	76	56	63	1,700	164	40	1.54	95.7	48.9	61/39	R.O.
38	2,649	64	53	38	—	—	—	—	93.9	47.5	50/50	R.P.
39	4,355	107	87	79	1,891	103	30	2.07	—	—	—	R.O.
40	2,000	46	41	36	—	—	—	—	89.6	52.0	—	R.P.
41	2,245	49	44	33	—	—	—	—	89.4	51.5	—	R.P.
42	3,170	78	78	74	—	—	—	—	—	—	—	A.
43	3,127	99	62	68	—	—	—	—	—	—	—	R.O.
44	1,645	46	67	76	—	—	—	—	—	—	—	R.O.
45	2,579	70	72	78	—	—	—	—	—	—	—	R.O.
46	1,585	34	32	27	—	—	—	—	—	—	—	R.P.
47	1,661	42	55	57	—	—	—	—	—	—	—	R.P.
48	2,268	52	31	27	—	—	—	—	—	—	—	R.P.
49	4,400	104	73	65	—	—	—	—	—	—	—	R.O.
50	2,128	55	49	61	—	—	—	—	—	—	—	R.O.
51	2,974	68	52	38	—	—	—	—	—	—	—	R.P.
52	2,224	52	58	54	—	—	—	—	—	—	—	R.P.
53	3,602	89	35	38	—	—	—	—	—	—	—	R.P.
54	2,680	69	51	56	—	—	—	—	—	—	—	R.O.
55	1,918	56	60	51	—	—	—	—	—	—	—	R.O.
56	4,258	102	78	69	—	—	—	—	—	—	—	R.O.
57	2,846	73	63	70	—	—	—	—	—	—	—	R.O.

*—Index of Intrapulmonary Mixing

A.—accepted for surgery

R.P.—refused surgery because of lung dysfunction

R.O.—refused surgery for reasons other than lung dysfunction

TABLE 3

REDUCTION OF VITAL CAPACITY AND MAXIMAL BREATHING CAPACITY AND APPEARANCE OF DYSPNEA IN 92 PATIENTS STUDIED SIX MONTHS POSTOPERATIVELY

Classified by Groups

Group	Number of Patients Studied	Number of Units Removed	Vital Capacity				Maximal Breathing Capacity				Post Operative Dyspnea
			Preoperative Mean	Postoperative Mean	Loss	Loss	Preoperative Mean	Postoperative Mean	Loss	Loss	
			ml.	*ml.*	*ml.*	*per cent*	*L./min.*	*L./min.*	*L./min.*	*per cent*	
I	10	3.3	3,838	3,605	233	6	124	115	9	7.2	12
II	10	3.0	4,290	3,664	626	15	129	115	14	10.8	1
III	12	3.9	3,098	2,759	339	11	75	65	10	13.0	2

TABLE 4

REDUCTION OF VITAL CAPACITY AND MAXIMAL BREATHING CAPACITY IN 92 PATIENTS STUDIED SIX MONTHS POSTOPERATIVELY

Classified by Units Removed

Units Removed	Number of Patients	Mean Preoperative Vital Capacity	Mean Postoperative Vital Capacity	Reduction in Vital Capacity	Mean Preoperative Maximal Breathing Capacity	Mean Postoperative Maximal Breathing Capacity	Reduction in Maximal Breathing Capacity
		ml.	*ml.*	*per cent*	*L./min.*	*L./min.*	*per cent*
1	20	3,718	3,507	6	123	119	3
2	9	3,811	3,564	6	113	112	0.9
3	9	3,633	3,291	9	110	101	8
4	38	3,954	3,615	9	119	110	8
5	10	3,938	3,688	6	111	98	12
6	4	3,204	2,724	15	117	89	24
7	2	2,964	2,568	13	79	73	8

DISCUSSION

Since the main object in this review is to outline a brief but efficient routine for the presurgical evaluation of the tuberculous subject, the results will be discussed in relation to the steps recommended in performing this routine.

Before doing so, it is perhaps wise to note first that the results in the present study could quite correctly be interpreted differently in other laboratories, since there is considerable variation in equipment, standard of surgical skill, and enthusiasm from hospital to hospital. Second, it should be noted that presurgical tests of lung function are only one of several factors to be considered in evaluating the patient. Perhaps of equal importance are the public health and economic aspects, the patient's personality, and the technical probabilities of the proposed operation.

With these facts in mind, the following routine is suggested in the evaluation of the surgical candidate, together with an interpretation of the data obtained:

1. Choice of a simple, efficient test of lung function as a screening test: Since the majority of patients presented for lung surgery have normal—or close to normal—function, there is no need to perform many complicated and time-consuming tests on all patients. Two simple tests (vital capacity and maximal breathing capacity) are performed in this hospital. Only one of these must be chosen, since there is no correlation between the two (5). This is well illustrated in the restrictive lesions of pulmonary tuberculosis in which purely pleural disease may reduce the vital capacity without affecting the maximal breathing capacity. Obstructive disease on the other hand (as illustrated in table 2) may provide a normal vital capacity but a low maximal breathing capacity. Briefly, the latter is believed to be the better test of lung function because it takes into consideration the elements of time and effort, while the vital capacity test does not (1). Indeed, the only argument against the maximal breathing capacity test is that it is a very vigorous test of lung function and perhaps contravenes the old view that the tuberculous lung should be rested. The timed vital capacity test (6) is less vigorous, measures the same mechanism as the maximal breathing capacity test, and could quite adequately replace the latter as the best single test of lung function.

2. Choice of a level above which no further tests are necessary: Assuming that the maximal breathing capacity test has been chosen as a screening test, a level must be decided on above which the patient will be approved for surgery and below which he will be studied further with more complicated tests of lung function. The choice by the present writers of a maximal

breathing capacity test which is 80 per cent of the predicted value is mainly empirical. This value, although suitable in this laboratory, might not apply to others, since the apparatus used to measure maximal breathing capacity varies considerably from laboratory to laboratory. The equipment used by Baldwin and associates (1) to express their normal values would no doubt give a lower value of maximal breathing capacity than the equipment used in this laboratory.

The 80 per cent level appears to be adequate since no postoperative shortness of breath was found in *Group I*, and only a single patient in *Group II* had questionable dyspnea attributable to lung dysfunction (table 1). The 3 deaths that occurred in the patients in these two groups whose maximal breathing capacity was greater than 80 per cent of the predicted value did not appear to be directly related to presurgical lung dysfunction. In this laboratory, this level is adequate; any possible revision could be a downward one. As shown in table 1, if this level is accepted, by this screening test, 352 (85 per cent) of the total of 417 patients should not require further study. This figure could be increased further if the surgical decision on those patients who were refused surgery for reasons other than lung dysfunction was made prior to performing the tests. A considerable number of the patients were refused surgery despite adequate lung function.

3. Choice of a level below which surgery is deemed inadvisable, regardless of the results of more intensive investigation: It is inadvisable to draw a rigid line in any aspect of medicine, since each patient must be evaluated on his own merits. The constant change in surgical outlook and the steady appearance of new techniques and equipment further substantiate this. Nevertheless, it seems advisable for every laboratory and every surgeon to have some conception of the degree of lung dysfunction below which one cannot expect to operate successfully. It is a level which is difficult to draw, one which will vary greatly from hospital to hospital, and one which must be flexible.

In table 2 it may be seen that the level of maximal breathing capacity below which the present writers believe surgery should not be performed is approximately 40 per cent of the predicted value. The lowest preoperative value was in a patient whose maximal breathing capacity was 45 per cent of the predicted value and in whom bronchospirometry showed absent function on the side on which pneumonectomy was planned. The patient was operated upon and died with pulmonary edema and bronchopleural fistula. This raised the question of complications in the group with poor function, which will be discussed later.

The surgeons did not list shortness of breath as a postoperative complication in any of the patients whose maximal breathing capacity was greater than 50 per cent of the predicted value, nor were there any deaths attributable to lung dysfunction in this group. Furthermore, the few instances of shortness of breath in those patients of *Group III* questioned six months or more after surgery leads to the conclusion that patients can certainly be operated on whose maximal breathing capacity is greater than 50 per cent of the predicted value. The writers' lack of experience below this level does not infer that it is unsafe. Again, it is possible that the value could be revised downward. With the use of tracheotomy, suction, a special respirator, and good postoperative care, Bjork and Engstrom (7) have performed a pneumonectomy on a patient whose maximal breathing capacity was 18 liters per minute, a figure which in an average man would be about 20 per cent of his predicted value.

4. Evaluation of other pulmonary function tests on those who require further investigation: The present report includes an important group of patients whose maximal breathing capacity falls between 40 and 80 per cent of the predicted value. The first question concerns the extent of necessary investigation. In order to answer this, it is necessary to consider the changes in pulmonary function caused by tuberculosis.

It has been pointed out that in some of the bilateral cases (*Group II*) there is a minor emphysema-like change characterized by a high ratio of residual volume to total lung capacity and a high index of intrapulmonary mixing. Those patients with a maximal breathing capacity of less than 80 per cent of the predicted value (*Group II*) showed more marked emphysema-like changes (*see* table 2). The pneumonectomy group was similar. Anno and Tomashefski (8) have demonstrated that the severity of these emphysema-like changes depends on the extent of the disease.

Other patterns of functional loss in tuberculosis are perhaps less important in regard to presurgical evaluation. McClement and associates (9) have shown that a defect in alveolar-capil-

lary diffusion may occur in hematogenous pulmonary tuberculosis, and it is generally accepted that pleural fibrosis can cause a purely restrictive lesion. In general, however, the type of functional loss in patients presented for surgical evaluation is one of an emphysema-like change, those with a low maximal breathing capacity having the most marked changes, as shown in table 2.

With this in mind, is there any indispensable information gained from measuring residual volume and arterial blood gases and performing bronchospirometry?

The maximal breathing capacity, as has already been stated, was all that was required in *Group I*. In the patients whose maximal breathing capacity was greater than 80 per cent of the predicted value and on whom bilateral surgery was planned (*Group II*), no useful knowledge appeared to be gained from measuring residual volume and arterial blood gases. Physiologically, the patients were similar to those in *Group I*. Bronchospirometry may, in some cases, aid the surgeon in deciding on which side to operate first. Theoretically, it is wise to interfere first on the side with the better function, since the poorly functioning contralateral side is at its maximum at that time. Practically, this knowledge seems to be rarely necessary. Of further significance was the fact that, of 61 patients who were presented as possible candidates for bilateral surgery (including 3 in *Group III*), only 9 actually had the surgery completed. There appeared, then, to be little reason for performing bronchospirometry in the patients of *Group II*.

In patients with a maximal breathing capacity of less than 80 per cent of the predicted value, it has been shown by the present writers that an emphysema-like state existed (*see* table 2). Baldwin and associates (10) have classified pulmonary emphysema according to the arterial blood gases and the presence or absence of right heart failure. There appears to be no correlation between these gases and residual volume. The severity of the emphysematous state, assuming that it exists in patients with a low maximal breathing capacity, is best reflected in the arterial blood gases. For this reason it is believed that the residual volume is not a necessary measurement in the presurgical estimation of lung function in the tuberculous patient. One can hardly escape the fact, however, that the arterial blood gases do provide useful, though perhaps not indispensable, information. In some centers this information may not play a part in the refusal or acceptance of a surgical candidate, but it provides the surgeon and anesthesiologist with vital information on the physiologic state of the body and allows them to modify and augment their therapy. The manometric method used for the analysis of these blood gases is not available in all thoracic units and, when available, the method is time consuming and requires a considerable amount of skill. Ear oximetry (5) and the carbon dioxide analyzer (11, 12) overcome some of these difficulties without losing too much of the accuracy of the Van Slyke procedure.

Interpretation of arterial blood analysis, of course, will vary from center to center. Anoxemia was not a contraindication to surgery in the experience of the present writers; an arterial oxygen saturation between 86.7 and 92 per cent was found in 11 of 37 cases studied in *Group III*. Six of the patients were operated upon uneventfully, and in those seen six or more months postoperatively there was only minor disability.

Carbon dioxide retention, a more advanced state of respiratory insufficiency, occurred in only one patient, a young male with "primary" emphysema. Three segments were removed and recovery was uneventful, without resource to special procedures such as tracheotomy or artificial respiration.

Woodruff and associates (13) find anoxemia and carbon dioxide retention no contraindication to thoracic surgery, and Bjork and Engstrom (7) have performed a pneumonectomy on a patient whose arterial carbon dioxide tension was as high as 73 mm. of mercury. The importance of carbon dioxide retention, however, should not be minimized. If the anesthesiologist and surgeon are aware of its presence, cognizant of the effects of respiratory depressants on its level, and have available the equipment to aid in its postoperative elimination, carbon dioxide retention can probably be handled adequately. It should be recognized, however, as an advanced state of respiratory insufficiency; and the effect of loss of any functioning lung tissue by surgery should be seriously considered, even though the means of eliminating carbon dioxide in the postoperative period are available.

In regard to the importance of bronchospirometry in those patients whose maximal breathing capacity was less than 80 per cent of the predicted value, one can only give an opinion. The present writers believe that it provided useful information

in the 15 per cent of patients who fell into this group. In only a few of these was the surgeon provided with the categorical answer he desired. In the remainder, the information added by bronchospirometry was indefinite, allowing the surgeon to base his decision solely on other factors, such as the necessity of the operation to the patient.

There is no definite level above or below which surgery can or cannot be performed. The decision depends on the total lung function and the ratio of function of one side to the other, both of which vary from patient to patient. Gaensler and associates (14) have illustrated the uses and interpretation of split-function tests, but in practically all instances criteria must be developed from personal experience.

5. Consideration of the probability of complications: It is not the purpose of the present writers to discuss surgical complications other than their incidence and the importance of considering their probability in the presurgical evaluation of the patient. This, as well as other considerations, will vary from hospital to hospital.

As shown in table 1, the fewest complications occurred in *Group I*, with a marked increase in *Group II*. The reason for this is not apparent. The patients in *Group III*, in whom the maximal breathing capacity was less than 80 per cent of the predicted value, had a higher incidence: 83 per cent of the 23 patients had complications, 26 per cent of them being multiple. Again, no attempt is made at an explanation other than to point out that there was no significant age difference (*see* table 1); there was no one complication which occurred more frequently in any one group; and an analysis of the number of lobes, segments, and wedges removed showed no preponderance in any one group. There is a suggsetion that the incidence of complications is higher in those who can least tolerate them, those with the poorest function.

6. Consideration of the probable loss of function after thoracic surgery: There are many difficulties in interpreting the results of functional change after resection. Various tests have been used, and these have been compared with the number of segments removed (15, 16). However, all segments are not of equal volume. Fleming (17) has taken this into account by the use of a unitage system. But even with a unitage system to express segmental volume the fact must be considered that removal of a shrunken tuberculous segment is not equivalent to removal of the same segment normally expanded. Other factors which make interpretation difficult are the variation in amount of normal tissue removed and the fact that a normal segment may expand and occupy the space left by the resected tissue, resulting in little or no loss of volume. It might be expected, then, that there would be a great variation of change in volume in a series of cases. Taylor and associates (16) have actually shown a wide range in the difference of the vital capacity before and after resection. This is also true in the patients of the present study. Of the 38 patients who had four units removed, the range was plus 648 ml. to minus 1,387 ml. However, when the mean reduction of vital capacity is considered, there is little variation, whether one or five units are removed. Taylor and associates (16) had similar results, attributing this to a "thoracotomy effect." The vital capacity is not a useful means of assessing loss of function postoperatively.

The maximal breathing capacity is not a measure of volume, as is the vital capacity; rather it is a test of muscular force, compliance of the lungs, and thorax and airway and tissue resistance (18). At first glance, then, one might not expect to find a correlation between this test and the number of units removed at operation. In table 4, however, it is certainly suggested that removal of one or two units produced no significant change in maximal breathing capacity, while removal of three to five units caused a reduction of 8 to 12 per cent. Taylor and associates (16) had similar results, using segments rather than units as the basis for loss of lung tissue. Mere loss of volume may not have as much effect on lung function as on the mechanics of breathing.

The reduction in maximal breathing capacity for *Groups I*, *II*, and *III* is shown in table 3. Since the volume removed by surgery averages more than two units, there is a 7 to 13 per cent reduction in maximal breathing capacity, the greatest reduction percentage occurring in *Group III*, from which a slightly greater amount of lung tissue had been removed than from the other two groups. It is in *Group III* that the greatest postoperative percentage reduction is found. The patients in this group could least afford to lose function.

It appears that the surgeon can expect little loss of function if he plans to remove only one or two units, but removal of three to five units can be expected to reduce the maximal breathing

capacity by 8 to 12 per cent. This reduction will affect lung function, as measured by the maximal breathing capacity, to a greater extent in those patients with the lower preoperative values.

An evaluation of the appearance or increase in shortness of breath six months postoperatively is a purely clinical means of estimating loss of function. Since dyspnea is an entirely subjective symptom, it is difficult to evaluate. Two patients stated that their preoperative shortness of breath was reduced by surgery, and 6 complained of this symptom despite a postoperative maximal breathing capacity which was 100 per cent or more of the predicted value. In 4 of these, the maximal breathing capacity was greater than the preoperative value. In only 2 cases in *Group III* was there a satisfactory explanation for the dyspnea, as shown by a considerable reduction in the maximal breathing capacity in patients whose preoperative values were 70 and 54 per cent of the predicted value.

It would seem fair to conclude that, with the exception of 2 cases, dyspnea was of little importance in the 92 cases studied six months or more postoperatively.

Summary

The results of the lung function tests on 417 patients with pulmonary tuberculosis presented for possible thoracic surgery have been reviewed, and a brief, practical routine for presurgical evaluation has been described.

It is believed that a surgical decision can be made on the basis of a screening test only (maximal breathing capacity) in at least 85 per cent of the cases. In the remainder, useful information is added by arterial blood gas analysis and bronchospirometry. Presurgical evaluation should take into account the possibility of postoperative complications and the estimated loss of function per unit of lung tissue removed. Those patients with the poorest function preoperatively have more complications and can least afford to lose more function.

Sumario

Sistema Práctico para la Justipreciación Prequirúrgica de la Función del Pulmón en la Tuberculosis Pulmonar

Después de repasar los resultados de las pruebas de la función pulmonar en 417 enfermos de tuberculosis pulmonar considerados para posible cirugía torácica, se describe un breve sistema práctico para la justipreciación prequirúrgica.

Parece que cabe tomar una decisión quirúrgica a base exclusiva de una prueba de despistaje (la capacidad respiratoria máxima) a lo menos en 85 por ciento de los casos. En el resto, aportan información útil el análisis de los gases de la sangre arterial y la broncoespirometría. La valuación prequirúrgica debe tomar en cuenta la posibilidad de complicaciones postoperatorias y la pérdida calculada de función por unidad de tejido pulmonar extirpado. Los enfermos que tienen la peor función postoperatoriamente muestran más complicaciones y son los que menos pueden tolerar más pérdida de función.

Resume

Un procédé routinier pratique pour l'évaluation préopératoire de la fonction pulmonaire dans la tuberculose du poumon

Les résultats des tests de la fonction pulmonaire chez 417 malades atteints de tuberculose pulmonaire ont été analysés et un procédé de routine pour l'évaluation préopératoire a été décrit.

Les auteurs estiment qu'une décision chirurgicale peut être prise en se fondant uniquement sur un test de dépistage (capacité respiratoire maxima), dans 85% des cas au moins. Dans les autres cas, l'analyse des gaz du sang artériel et la bronchospirométrie constituent des moyens d'information supplémentaires. L'évaluation préopératoire doit tenir compte de la possibilité de complications postopératoires et de la perte de fonction prévue par unité de tissu pulmonaire réséqué. Ceux des malades à fonction pulmonaire la plus altérée avant l'intervention ont présenté le plus de complications, alors qu'ils étaient moins aptes à subir un nouveau déficit de cette fonction.

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MICROSCOPIC ENUMERATION OF MYCOBACTERIA IN PULMONARY LESIONS OF TUBERCULOUS MICE[1]

Effect of Prior BCG Vaccination

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Introduction

The protective effect of BCG vaccination in mouse tuberculosis can be established by comparing the numbers of viable bacilli detectable in the organs of normal and vaccinated animals at various periods of time after virulent infection. The techniques used in this laboratory for such viable population studies have been previously described (1 and 2). In the present report, another test is described of immunity based on the enumeration of the bacilli that can be seen in stained sections of the lungs in infected mice. Some results comparing the results of these two techniques are also presented.

Methods

Different preparations of BCG vaccine were used, as indicated in the text and tables.

A mouse-adapted culture of H37Rv was used for the virulent infection tests. This culture was maintained in the virulent state by recovering it at intervals from the spleens of infected mice; it was otherwise subcultured in Tween®-albumin medium. The cultures were eight to ten days old at the time of the infection tests.

Both the vaccinating dose of BCG and the virulent infection were administered by the intravenous route in a final volume of 0.2 ml. by appropriate dilutions with physiologic saline.

The tests were carried out with albino mice obtained from two colonies maintained near Paris, but derived from one of the so-called "Swiss" strains used at the Rockefeller Institute (3). The mice were weaned at approximately four weeks of age and from then on were fed commercial pellets supplemented with cabbage and carrots. They were vaccinated when approximately four weeks old, at which time they weighed 10 to 15 gm.

In general, the animals were sacrificed three weeks after virulent infection; less commonly, after six weeks. The results have been essentially the same with these two different intervals between virulent infection and sacrifice.

The size of the virulent infective dose proved to be of great importance for the success of the technique. When the dose was too large (approximately that used in survival tests), the bacilli in the lungs were so numerous and occurred in clumps of such enormous volume that microscopic enumeration was all but impossible. In practice, an inoculum of 0.2×10^{-3} ml. proved to be a satisfactory dose for the Tween-albumin culture of H37Rv employed in this laboratory.

In the present study it seemed of interest to determine comparatively on the same animal the numbers of tubercle bacilli detectable in the lungs by microscopic technique and the numbers of viable units capable of giving rise to colonies on appropriate culture media. For this purpose, the following procedure has been used in all of the experiments reported here.

For each animal, the right lung was selected for viable population studies. Lung homogenates were prepared and proper dilutions were inoculated on Jensen's egg medium according to the techniques previously mentioned (1 and 2). The left lung—which usually contained larger numbers of bacteria than the right lung—was fixed in Bouin's fluid for three days, embedded in paraffin, and sectioned in its entirety to a thickness of 5 μ. It has been the practice to section the paraffin blocks along a horizontal plan corresponding to the verticofrontal orientation in man. Thus, each section included both the apex and the other lobe of the lung, the first sections corresponding to the dorsal region of the mouse, and the last sections to the ventral region.

The sections were stained by the Ziehl-Neelsen technique for forty-five minutes at 56°C. They were decolorized with lactic acid (Häuser's technique) and counterstained with malachite green.

Although the whole lung was sectioned, only one section of twenty was usually examined. Each twentieth section was placed on a slide according to the order of cutting, three sections per slide, thus giving 10 to 20 slides per lung. By numbering the slides consecutively, it was thus possible to have an accurate picture of the anatomic location of the microscopic field under study. Ten slides per lung were studied intensively: one section per slide, one lesion per section. As the numbers of slides per lung usually exceeded 10, the first and the last few were ex-

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TABLE 1

EFFECT OF BCG ON NUMBERS OF PULMONARY LESIONS IN MICE THREE WEEKS AFTER VIRULENT INFECTION

Treatment of Animals		Numbers of Lesions in Lungs of Individual Animals											
BCG*	H37Rv†	Upper ⅔ of Lungs‡						Ventral Zone of Lungs‡					
Yes	No	0	19	24	49	49	51	0	6	3	0	35	18
No	Yes	16	58	70	71	139		10	49	40	12	28	
Yes	Yes	19	54	104	114	238	644	10	21	34	31	103	192

* 7,000 viable units intravenously

† 0.2×10^{-3} ml. of an 8-day-old culture, intravenously

‡ Numbers arranged in increasing order for the upper region; figures for ventral zone arranged in the same order as for the upper region

TABLE 2

EFFECT OF BCG VACCINATION ON NUMBERS OF MYCOBACTERIA PER PULMONARY LESION IN MICE THREE WEEKS AFTER VIRULENT INFECTION

Treatment of Animals		Number of Bacteria Per Pulmonary Lesion											
BCG*	H37Rv†	Upper ⅔ of Lungs‡						Ventral Zone of Lungs‡					
No	Yes	16	127	202	450	839		42	32	299	13	453	
Yes	Yes	1	2	4	8	10	15	1	1	1	1	10	4

* 7,000 viable units intravenously

† 0.2×10^{-3} ml. of an 8-day-old culture, intravenously

‡ Numbers arranged in increasing order for the upper region; figures for ventral zone arranged in the same order as for the upper region

cluded whenever possible. A minimum of ten lesions was examined for each lung.[3]

In order to locate the lesions, each section was first examined at low microscopic power; then the individual lesions were examined under the oil immersion lens in order to count the mycobacteria. It was found, of course, that large lesions often extended over several consecutive sections, but an attempt was made to avoid counting mycobacteria in the same lesion more than once. To this end, microscopic examination was begun at a certain orientation on the first slide, and the following slides were examined by turning clockwise for half of the mice in one group and counterclockwise for the other half.

For reasons to be discussed later, the results to be emphasized in the present paper correspond, not to the numbers of lesions, but to the numbers of mycobacteria seen per individual lesion. It must be acknowledged that the comparison of results between vaccinated and control animals has presented some difficulty arising from the fact that the dispersion of numbers within each group was too great to justify the calculation of averages (tables 2 and 3). On the advice of Dr. D. Schwartz (4), therefore, the results are presented by the test of rank according to which the values obtained for each animal are arranged in increasing order of magnitude. This presentation reveals that in the experiment illustrated in table 2 there was little, if any, overlapping between the numbers of bacilli in the vaccinated and nonvaccinated groups. In other words, the smallest number of bacilli in the lesions of the control group was larger than the largest number in the vaccinated group—evidence of a high degree of significance in the results. Even in cases in which there was some overlapping, this technique permitted the determination of the degree of significance of the differences observed.

[3] The technique has been modified slightly in more recent experiments. Instead of examining one section of twenty, we retain only two consecutive sections of eighty at regular intervals. One of these two sections is examined, and the other one is held in reserve in case of defective staining of the first.

RESULTS

Localization and numbers of lesions: As is well known, tuberculous lesions in man are not homogeneously distributed, not even in generalized miliary tuberculosis; they are usually more prevalent in the apex and in the mid-lung region. In contrast, as pointed out by Medlar (5), the lesions in the bovine, the dog, the rabbit, and the guinea pig are chiefly in the dorsal region,

whereas they are often at the base of the lung in the bat, which usually lives head down.

Whatever the reasons which account for these peculiarities of anatomic distribution in the various species, a similar law appears to govern the distribution of pulmonary lesions in the mouse. As seen in tables 1 and 2, the lesions in the dorsal part of the lung (upper two-thirds) outnumber somewhat those in the lower part; moreover, they tend to contain more bacteria. The upper and middle third do not differ greatly in either respect.

Effect of BCG vaccination on the numbers of mycobacteria per lesion: Early in the course of this study, it became apparent that the number of pulmonary lesions in mice infected with H37Rv was not decreased by prior BCG vaccination (*see* table 1). In contrast, the numbers of mycobacteria detectable by microscopy in each lesion revealed marked differences between the two groups (tables 2, 3, 4, 5). It was for this reason that emphasis was placed on determining the numbers of mycobacteria per lesion in an attempt to evaluate the comparative effectiveness of different BCG vaccines. The details of the technique used have been described under Methods.

The use of this microscopic technique will now be illustrated by two experiments designed to compare the activities of two different batches of BCG vaccine.

In one experiment, mice were vaccinated with either one of two different batches of BCG which differed in the age of the culture from which the vaccine had been prepared. In one case the BCG culture was thirteen days old when the cells were harvested; in the other the culture was twenty days old. The vaccinating doses were so arranged as to contain approximately the same number of viable units (2,800 for the 13-day-old culture, 2,600 for the 20-day-old). Control mice received saline instead of vaccine. Six weeks after vaccination some of the animals received a virulent infection in the form of 10,000 viable units of H37Rv.

Three weeks after the virulent infection, all animals were sacrificed and the numbers of bacilli detectable per pulmonary lesion were determined by the microscopic technique described under Methods. The numbers of viable mycobacteria

TABLE 3

EFFECT OF BCG VACCINATION ON NUMBERS OF MYCOBACTERIA IN LUNGS OF MICE THREE WEEKS AFTER VIRULENT INFECTION

Treatment of Animals		Number of Mycobacteria‡ as Determined by									
BCG*	H37Rv†	Microscopy/Lesion					Cultivation on Egg Medium				
No	Yes	174	237	558	1,208	2,220	35	40	25	>100	>100
Yes	Yes	3	4	10	41	56	10	1	5	0	2

* 8,000 viable units of lyophilized BCG, intravenously
† 3,200 viable units of lyophilized BCG, intravenously
‡ To be multiplied by 6,000 for whole organ

TABLE 4

COMPARATIVE EFFECTS OF TWO DIFFERENT BCG VACCINES ON NUMBERS OF MYCOBACTERIA IN LUNGS OF MICE THREE WEEKS AFTER VIRULENT INFECTION

Treatment of Animals			Numbers of Mycobacteria in Lungs as Determined by									
BCG-A*	BCG-B†	H37Rv‡	Microscopy/Lesion					Cultivation on Egg Medium§				
Yes	No	No	0	0	0	1	17	1	0	0	1	1
No	Yes	No	1	1	4	35	53	0	0	0	0	0
No	No	Yes	100	229	316	330	565	32	>100	55	55	55
Yes	No	Yes	1	1	2	3	3	1	2	15	1	4
No	Yes	Yes	2	10	27	66	153	11	7	14	60	12

* Lyophilized vaccine made from 13-day-old BCG culture, 2,800 viable units injected
† Lyophilized vaccine made from 20-day-old BCG culture, 2,600 viable units injected
‡ 10,000 viable units injected six weeks after vaccination; animals sacrificed three weeks after virulent infection
§ Numbers to be multiplied by 600 for whole organ

TABLE 5

PROTECTIVE EFFECTS OF TWO DIFFERENT BCG VACCINES AGAINST MOUSE TUBERCULOSIS MEASURED BY THREE DIFFERENT TECHNIQUES

Treatment of Animals			Average Survival Time	Numbers of Mycobacteria in Lungs as Determined by									
				Microscopy/Lesion					Cultivation on Egg Medium§				
BCG-A*	BCG-B(†)	H37Rv‡	(*days*)										
Yes	No	No	—	0	0	1	3		0	0		0	0
No	Yes	No	—	1	1	1	4	5	0	0	0	0	0
No	No	Yes	17	12	33	277	308	∞	5	23	>100	11	>100
Yes	No	Yes	20.3	0	0	1	1	32	0	0	0	0	
No	Yes	Yes	16.5	0		40	67		0	1	7		

* BCG, fresh, 2×10^{-3} mg., 23,000 viable units

† BCG, lyophilized, 2×10^{-3} mg., 3,600 viable units

‡ H37Rv administered four weeks after vaccination; 1,000 viable units for enumeration of colonies; animals sacrificed three weeks after infection; 1,000,000 viable units for survival test

§ Numbers to be multiplied by 12,000 for whole organ

capable of giving rise to colonies were also determined by plating on egg media appropriate dilutions of lung homogenates (1 and 2). Thus, animals which received BCG alone were sacrificed nine weeks after vaccination, those which received H37Rv alone were sacrificed three weeks after virulent infection, and those which received both BCG and H37Rv were sacrificed nine weeks after vaccination and three weeks after virulent infection (table 3).

The results presented in table 3 show that the numbers of mycobacteria detectable by microscopy in each lesion were very much larger in nonvaccinated animals infected with H37Rv than in the vaccinated groups. As vaccination with BCG alone resulted in the presence of a certain number of bacteria per lesion, the difference in the multiplication of H37Rv between the vaccinated and nonvaccinated groups is probably even greater than appears from the results presented in table 3. Both preparations of BCG vaccine elicited a high degree of protection. However, the results strongly suggest that the vaccine made from the 13-day-old cells was more active than the one made from the 20-day-old cells. Similar conclusions can be derived from the results of enumeration of colonies produced on egg media by inoculation of lung homogenates.

Another experiment was designed to compare the protective activity of two samples of vaccine, one of which had been lyophilized before use.

Both samples were derived from the same BCG culture. One was lyophilized in a glucose solution; the other was kept untreated in Sauton medium diluted 1:4 with physiologic saline. Although the same weight dose of vaccine was used in both cases (2×10^{-3} mg.) the lyophilized lot contained a much smaller number of viable units than the untreated lot at the time of vaccination—3,600 and 23,000, respectively.

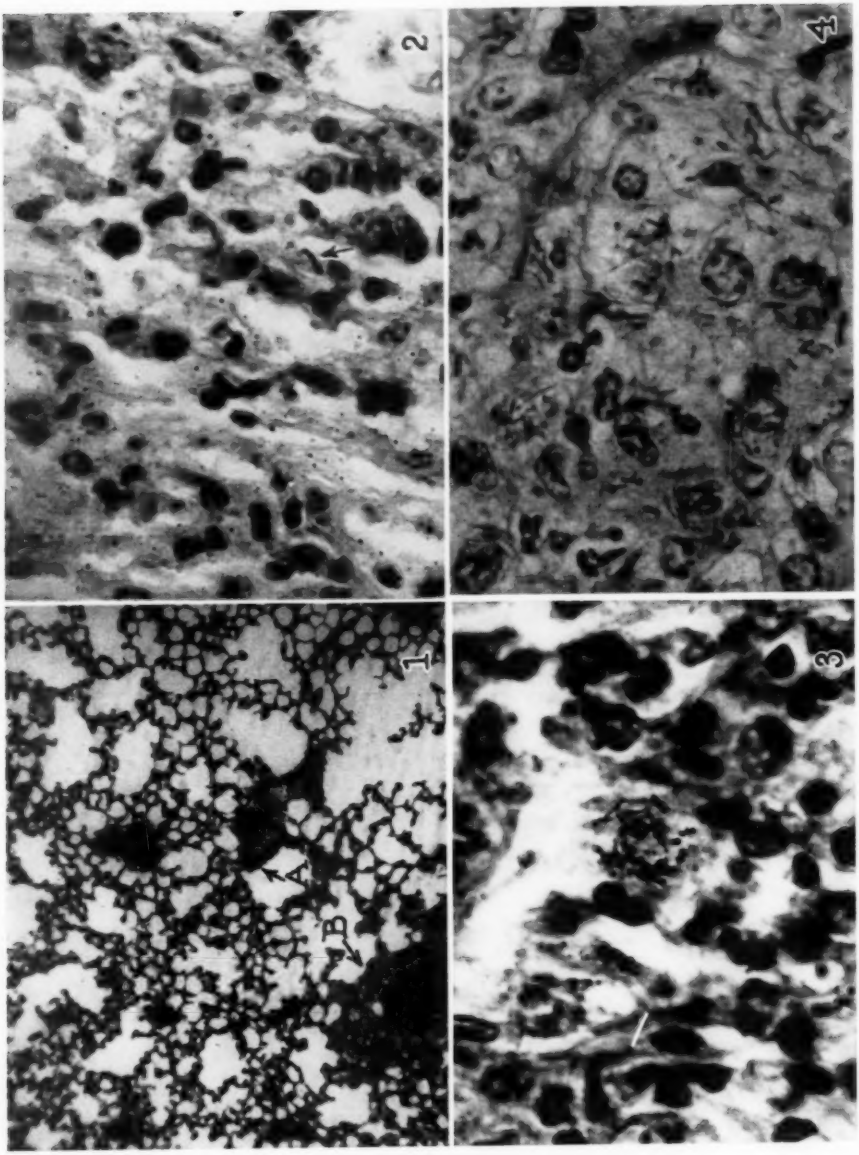
Six weeks after vaccination some of the vaccinated animals as well as nonvaccinated controls were infected with H37Rv. Two infective doses were used. Animals receiving 1,000 viable units of H37Rv were sacrificed three weeks after infection and their lungs were studied with regard to both their content in mycobacteria detectable by microscopy and the numbers of organisms capable of producing colonies on egg medium. Other animals received a much larger infective dose (0.02 ml. of Tween-albumin culture corresponding to approximately 1,000,000 viable units) and these were allowed to die of tuberculosis in order to determine the effect of vaccination on survival time (table 5).

The results presented in table 5 confirm those in table 4: they show that infected animals which received either one of the two BCG vaccines contained far fewer mycobacteria per pulmonary lesion than did those which received H37Rv without vaccination. As will be noticed also, lyophilization of the vaccine appeared to have reduced somewhat its protective efficacy.

The results of tests of survival time and of enumeration of colonies on egg medium were compatible with those derived from microscopic examination of lung tissue.

DISCUSSION

Several kinds of tests are available to evaluate the protective effectiveness of BCG vaccines against mouse tuberculosis. The determination of survival time following virulent infection is the most obvious of these tests, but it has little merit



FIGS. 1–4.

other than its technical simplicity. As is well known, the survival time is profoundly influenced by many factors unrelated to immunity; the differences that it reveals between vaccinated and nonvaccinated groups are never large, and the interpretation of their mechanisms is all but impossible.

The other two tests considered in the present paper are based on a study of the numbers of bacilli present in the lungs at a given time after virulent infection; these numbers are determined either by quantitative bacteriologic techniques of cultivation on appropriate culture media, or by microscopic examination of stained sections of the lung. Both tests have two superior qualities in common in determining survival time, namely, both involve the use of small infective doses and both provide useful information as to the fate of the bacilli in the tissues.

The pathologic character of tuberculosis in the mouse lends itself well to the microscopic study of the numbers of bacilli in the pulmonary tissue. In this animal the disease presents predominantly a monomorph histologic picture. When the experimental infection is carried out under the proper conditions, the great majority of bacilli are found within the nodules, they are intracellular, and they rarely occur in large aggregates. For all of these reasons it is relatively easy to evaluate their abundance by microscopic examination.

Assessment of the numbers of tubercle bacilli in sections of the lung has been frequently used for the analysis of mouse tuberculosis (6–13). In the present study an attempt has been made to refine this technique and to render it more quantitative, particularly by using very small infective doses (1,000 to 10,000 viable units of H37Rv), and by determining the numbers of bacilli per pulmonary lesion rather than on the whole surface of the organ.

While the present paper is primarily concerned with the description and not the applications of this technique, it may be worth while to discuss briefly some of the results that it has yielded so far.

As shown in tables 4 and 5, the different samples of BCG vaccine were found to differ in their ability to protect mice against tuberculosis. Aging of the BCG bacilli and lyophilization resulting in a partial loss of their viability had the effect of decreasing the protective activity of the vaccine. Of particular relevance to the present study is the fact that this decrease in activity could be detected by the three methods used for the evaluation of the vaccine. It is not yet possible to affirm that the three methods would always give concordant results when used for the evaluation of vaccines differing in other characters, since discrepancies might result from the fact that these methods appraise different aspects of the experimental disease. As already mentioned, the survival time is certainly influenced by many factors other than those having to do with the numbers of bacilli in the organs. More similarity can be expected between the patterns of results obtained by the microscopic technique and by cultivation of lung homogenates on culture media. In the present study, in fact, this similarity was great with regard to the minimal dose of BCG capable of eliciting a significant level of protection. However, differences in results are bound to result from limitations inherent in, and peculiar to, each one of the techniques. For example, there is at present no way of knowing whether the bacilli visible on stained lung sections are dead or viable, or of knowing, on the other hand, whether colonies developing on culture media come from single cells or from clumps of varying sizes. It would be of great interest to follow the relative changes that occur in the numbers of visible cells and of viable cells in the course of the experimental disease, as this comparative study would bid fair to throw additional light on the fate of the bacilli *in vivo*.

The microscopic study of infected lungs has brought out another fact which is likely to have

FIGS. 1–4.

FIG. 1. Normal mouse infected with virulent H37Rv. Low magnification (× 28) reveals two pulmonary lesions: one small (*A*) near a vessel, and one large (*B*).

FIG. 2. Mouse vaccinated with BCG, but not superinfected. High magnification (× 800) reveals only one bacillus in the whole field.

FIG. 3. Mouse vaccinated with BCG, then superinfected with virulent H37Rv. High magnification (× 800) reveals approximately 15 bacilli per field.

FIG. 4. Normal mouse infected with H37Rv. High magnification (× 800) reveals numerous bacilli (approximately 100 per field).

(*These microphotographs were taken by Mr. Vergé, Centre National de Recherches Zootechniques, Jouy en Josas, France.*)

a bearing on the mechanism of BCG immunity. Whereas the numbers of bacilli per pulmonary lesion in well-immunized mice are much smaller than those found in control animals, immunization does not appear to decrease the numbers of these lesions in the lungs. This fact is compatible with earlier findings that, however effective the immunizing process, and even when the virulent infective dose is very small, some degree of multiplication of virulent bacilli always takes place in the immunized animals (1*b*). In other words, it appears that immunization with BCG does not prevent the establishment of tubercle bacilli in the tissues. The significance of these findings and their relevance to tuberculosis in man have been greatly increased by facts recently reported by Cohen, Davis and Middlebrook (14, 15). In their words, "Using the airborne infection method, we have observed that BCG vaccination, under the best conditions with guinea pigs, rabbits, and mice, does not protect against infection . . . .It does not protect against the early multiplication of tubercle bacilli." Thus, several independent studies lead to the conclusion that, whether the infective dose is administered intravenously or in the form of aerosols, BCG vaccination does not prevent the establishment of the bacilli and their initial multiplication. Instead, its effect is exerted in the form of changes in the course of the infectious process.

Summary

A technique is described for the microscopic enumeration of lesions and of mycobacteria per lesion in the lungs of mice infected with virulent tubercle bacilli. This technique has been used to study the effect of BCG vaccination on mouse tuberculosis.

The results reveal that BCG vaccination does not decrease the number of pulmonary lesions elicited by virulent infection, whereas it can depress profoundly the numbers of bacilli detectable by microscopy in each lesion.

It is suggested that the microscopic enumeration of bacilli per lesion provides a measure of the protective activity of BCG vaccines. The results obtained by this technique with several different samples of vaccine are compared with those given by survival tests in mice and by enumeration of the viable bacilli in infected tissues.

A discussion is presented of the mechanisms of the protective effect of BCG. The conclusion is reached that the immune process elicited by BCG does not prevent the establishment and initial multiplication of virulent bacilli in the tissues, but only interferes with the subsequent course of the infectious process.

Sumario

Enumeración Microscópica de las Micobacterias en las Lesiones Pulmonares de los Ratones Tuberculosos. Efecto de la Vacunación Anterior con BCG

Descríbese una técnica destinada a la enumeración microscópica de las lesiones y de las micobacterias por lesión observadas en los pulmones de los ratones infectados con bacilos tuberculosos virulentos. Esta técnica ha sido empleada para estudiar el efecto de la vacunación con BCG sobre la tuberculosis murina.

Los resultados revelan que la vacunación con BCG no hace disminuir el número de lesiones pulmonares provocadas por la infección virulenta, en tanto que puede deprimir profundamente el número de bacilos descubribles con la microscopía en cada lesión.

Apúntase que la enumeración microscópica de los bacilos por lesión ofrece una medición de la actividad protectora de las vacunas BCG. Compáranse los resultados obtenidos con esta técnica con varias distintas muestras de vacuna con los rendidos por pruebas de sobrevivencia en los ratones y por la enumeración de los bacilos viables en tejidos infectados.

Preséntase una discusión de los mecanismos del efecto protector del BCG. Llégase a la conclusión de que el proceso inmune provocado por el BCG no impide el establecimiento y multiplicación inicial de bacilos virulentos en los tejidos, y que sólo afecta la evolución subsiguiente del proceso infeccioso.

Resume

Numération microscopique des mycobactéries dans les lésions pulmonaires de souris tuberculeuses. Effet de la vaccination antérieure par le BCG

Description d'une technique pour la numération microscopique des lésions et des mycobactéries, par lésion, dans les poumons de souris inoculées avec des bacilles tuberculeux virulents. Cette technique a été utilisée pour étudier l'effet de la vaccination par le BCG sur la tuberculose de la souris.

Les résultats révèlent que la vaccination BCG ne réduit pas le nombre des lésions pulmonaires provoquées par l'infection virulente, alors qu'elle peut réduire considérablement le nombre des

bacilles décelables microscopiquement dans chaque lésion.

Il est suggéré que la numération microscopique des bacilles par lésion procure une mesure de l'activité protectrice des vaccins BCG. Les résultats obtenus par cette technique avec plusieurs échantillons différents du vaccin sont confrontés avec ceux donnés par les tests de survie chez la souris et par la numération des bacilles viables dans les tissus infectés. Des commentaires sur les mécanismes de l'effet protecteur du BCG sont présentés. Les auteurs sont amenés à conclure que le processus immun déterminé par le BCG n'empêche pas l'établissement et la multiplication initiale de bacilles virulents dans les tissus, mais qu'il intervient seulement dans l'évolution ultérieure du processus infectieux.

Addendum

In a study which has appeared since the present paper was submitted for publication, Suter and Strain have provided evidence that the antituberculous immunity induced in mice by *Mycobacterium X* can be demonstrated by a method similar to the one described above (16).

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CHEMOTHERAPY OF TUBERCULOSIS[1]

IV. Stability of Hydrazones of Isoniazid in Biologic Fluids

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INTRODUCTION

The chemotherapeutic activity of a number of hydrazones of isoniazid has been described (1–3) and it has been established that their *in vitro* and *in vivo* activity is equal to that of isoniazid. Their mode of action is also similar since cross-resistance between isoniazid and hydrazones can be readily demonstrated (4). Whether the action of the hydrazone on tubercle bacilli is due to the intact molecule or to isoniazid has not been established. However, *in vivo* the hydrazones are subject to possible breakdown to isoniazid before they have had the opportunity of coming in contact with the infecting organism.

In a previous study (5), serum concentrations of Verazide, 3:4-dimethoxybenzal *iso*nicotinyl hydrazone, were studied in a number of volunteers and no indication of breakdown to isoniazid was obtained. More recently, however, during the progress of a clinical trial (1), unexpectedly low serum concentrations of Verazide were obtained (<0.5 γ per ml.) in patients who clearly showed good response to therapy. This result strongly suggested that Verazide liberated isoniazid *in vivo*, and this view has now been confirmed by chemical estimation. Accordingly, it was decided to investigate the stability of Verazide and some other hydrazones of clinical interest in body fluids and tissue homogenates.

In the present paper, it will be shown that the half-life of Verazide, Phtivazid®, and Nupasal-213® in blood and tissue homogenates is two to four hours. One product of their breakdown *in vivo* is isoniazid, which would account for the good clinical response seen in hydrazone therapy.

MATERIALS AND METHODS

The hydrazones used in this study have been described (4): Verazide, 3:4-dimethoxybenzal *iso*nicotinyl hydrazone; Phtivazid (or vanizide), 3-methoxy-4-hydroxybenzal *iso*nicotinyl hydrazone; Nupasal-213 (or Salizid®), 2-hydroxybenzal *iso*nicotinyl hydrazone.

The estimation of the hydrazones in citrated blood, serum, and tissue homogenates followed, with some modification, the method previously described (5). By using the differential solubility of these hydrazones in ethyl acetate, it was possible to estimate any one and isoniazid in the same sample of fluid. Briefly, a 3-ml. sample of blood, serum, or tissue homogenate containing the aromatic aldehyde hydrazone was extracted with 5 ml. of ethyl acetate (analytic reagent quality) for fifteen minutes by gentle horizontal mechanical agitation in glass-stoppered Pyrex tubes. The ethyl acetate phase was separated by centrifugation in the case of serum, plasma, and tissue homogenates, but with citrated blood the two phases readily separated. The absorption of the ethyl acetate extract was read at a wavelength of 330 mμ against pure ethyl acetate (AR) in 1-cm. silica cells using a Unicam model SP500 spectrophotometer. Extracts of normal fluids with ethyl acetate showed very low and consistent absorption at 330 mμ. The concentration of the hydrazone in the extracted fluid was calculated from standard curves calibrated for each fluid in the following manner. A known amount of hydrazone was added to the appropriate biologic fluid and was immediately extracted with ethyl acetate as above. The optical density of the ethyl acetate extract at 330 mμ was plotted against the known concentration of hydrazone, and a standard curve was constructed. It was found that Beer's Law was obeyed over the range of concentrations used with a deviation of plus-minus 0.25 γ per ml. The recovery of added hydrazone in the ethyl acetate phase was 95 to 100 per cent.

The isoniazid released from the breakdown of the hydrazones was estimated by the Kelly and Poet method (6) after extraction with ethyl acetate. As a check on the specificity of this method in these experiments, a modified picryl chloride technique was also used (7, 8). In general, the results obtained by the two methods agreed. A standard curve was prepared for the Kelly and Poet technique by adding known amounts of isoniazid to the biologic fluid, extracting with ethyl acetate, and estimating the isoniazid left in the aqueous phase.

A more detailed analysis of Verazide breakdown was sought by using radioactive Verazide in fresh human plasma (approximately 40 γ per ml.). The residual drug was estimated in the ethyl acetate extract spectrophotometrically (as above) and by radioactive count, and the aqueous phase was examined quantitatively for isoniazid and

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*iso*nicotinic acid by a modification of the cyanogen bromide method described by Rubin and associates (9). The modification involved precipitating the proteins by diluting the plasma with water and adding trichloracetic acid, centrifuging, and adjusting the pH to 8.3 with 10N. NaOH.

Unless otherwise stated, the various biologic materials were used within twenty-four hours of collection or preparation. They consisted of citrated human blood, human and guinea pig serum and plasma, and homogenates of various guinea pig tissues. The latter were prepared by grinding the tissue in a pestle and mortar with powdered glass and suspending in M/15 phosphate buffer, pH 7.2, in a concentration of 20 per cent by weight.

The human subjects on whom blood concentrations were done following a single dose of the various drugs were all normal male adults.

Results

An experiment (typical of many) indicating the rate of disappearance of Verazide in blood and serum is set forth in table 1. It will be noted that the drug is stable in phosphate buffer solution but disappears quite rapidly in blood and serum. In fact, after eight hours' incubation no Verazide was detectable if the original concentration was 10 γ per ml. or less.

In table 2 the rate of disappearance of Verazide in various media is expressed as a half-life, which was calculated from a graph charting residual Verazide in solution against time. It will be seen that the half-life of the drug was significantly prolonged only when the serum was diluted more than 50 per cent or when the proteins were completely removed by trichloracetic acid precipitation. In the latter case, 2 ml. of 30 per cent acid were added to 4 ml. of serum, filtered, neutralized, and diluted to 8 ml. in M/15 phosphate buffer prior to adding Verazide. Destruction of complement by mild heat treatment (56°C. for thirty minutes), or denaturation and coagulation of protein by boiling for one hour, or removal of electrolytes by dialysis did not affect the hydrolytic activity of the serum. The tissue homogenates were all uniformly active, and it could be assumed that, *in vivo*, Verazide would disappear in approximately four to six hours irrespective of its distribution in the animal body.

Furthermore, the rate of disappearance of Verazide from serum at various temperatures is shown in figure 1. The reaction is sensitive to temperature, since at 4°C. the drug is fairly stable in this medium for at least twenty-four hours, unstable at 37°C., and of intermediate stability at room temperature.

A similar series of experiments was then done with the other hydrazones of clinical interest. The results have been summarized in table 3, which shows that Nupasal and Phtivazid are equally unstable in serum or whole blood and disappear at a slightly faster rate than that observed for Verazide. It would thus appear that these hydrazones also are chemically unstable *in vivo* and that their rate of disappearance precludes the possibility of direct action of the intact molecule against the tubercle bacillus. This view was further confirmed by examining the hydrazone content of plasma from persons given single doses of the various hydrazones (*see* table 5).

In order to determine whether the disappearance of hydrazone could be accounted for in the appearance of isoniazid and *iso*nicotinic acid, the fate of radioactive Verazide in human plasma was followed. To this end a high concentration of the drug was used, so that the products could be estimated quantitatively by methods already described. The results with four different specimens of human plasma are recorded in table 4.

As may be seen, the findings were consistently uniform and give a clear picture of the distribution of the nitrogenous products of Verazide

TABLE 1

Stability of Verazide in Various Fluids

Concentration 10 γ per Ml.: Temperature 37°C.

Fluid Vehicle	Per Cent Verazide Remaining After (Hours)							4 Days
	0	1	2	4	6	8	24	
Phosphate buffer, pH 7.2	100	100	100	100	100	100	100	100
Human blood (citrated), pH 7.3	100	82	67	44	25	0	0	0
Human serum, pH 7.8	100	74	50	11	8	0	0	0
Guinea pig serum, pH 7.7	100	60	30	0	0	0	0	0

breakdown. In round figures, 60 per cent of the Verazide was hydrolyzed to isoniazid, 25 per cent appeared as *iso*nicotinic acid, and 15 per cent was unchanged. The relatively high residual Verazide is attributed to the high initial concentration of the drug and the formation of veratric aldehyde. Evidence for the latter was the shift in the absorption maximum from 330 mμ to 315 mμ with time, indicating the formation of a substance absorbing at shorter wavelength than Verazide (veratric aldehyde λ_{max} = 308 mμ). On the evidence, it is concluded that the disappearance of Verazide and other hydrazones of isoniazid from biologic fluids is due to hydrolysis to isoniazid which, in turn, is partially converted to *iso*nicotinic acid.

Finally, an attempt was made to measure the hydrazone and isoniazid concentrations in the plasma of healthy adults following a single dose of 10 mg. per kg. of body weight. The results are shown in table 5. It will be seen that no detectable hydrazone was found, but in most cases some isoniazid was detected. The findings confirm the *in vitro* results described above.

TABLE 2

HALF-LIFE OF VERAZIDE IN VARIOUS BIOLOGIC FLUIDS

Concentration 10 γ per Ml.: Temperature 37°C.

Biologic Fluid	Half-Life of Verazide in Hours
Human serum, undiluted	2.0
Human serum, 50% in saline	2.5
Human serum, 25% in saline	4.0
Human serum, 12.5% in saline	9.0
Human serum, 6.25% in saline	18.0
Human serum,* 50% (heated 30 min., 56°C.)	2.5
Human serum,* 50% (heated 1 hour, 100°C.)	2.5
Human serum,* 50% (dialyzed)	2.5
Human serum, (treated with trichloracetic acid)	>4 days
Guinea pig liver suspension, pH 7.2	2.25
Guinea pig muscle suspension, pH 7.2	2.25
Guinea pig spleen suspension, pH 7.2	2.75
Guinea pig bowel suspension, pH 7.2	4.5

* Diluted in M/15 phosphate buffer, pH 7.2.

TABLE 3

STABILITY OF HYDRAZONES OF ISONIAZID IN HUMAN BLOOD AND SERUM

Concentration 10 γ per Ml.: Temperature 37°C.

Hydrazone	Half-life in Hours	
	Serum	Citrated Whole Blood
Nupasal (Salizid)	0.75	0.75
Phtivazid	1.25	1.75
Verazide	2.0	3.5

DISCUSSION

In recent years, interest in the chemotherapeutic activity of the hydrazones of isoniazid has been generated because of their undoubtedly low animal toxicity and the fact that their molecules are not susceptible to acetylation *in vivo*. That they are chemotherapeutically active in human (1–3) and experimental tuberculosis (4, 10) is unquestioned. However, it has not been clear whether they are acting *in vivo* as hydrazones or whether their activity is due to the liberation of isoniazid. Although Grebennik (11) described the distribution of Phtivazid in organs and tissues of living animals, it is evident from a perusal of his method of estimation that isoniazid and not Phtivazid was measured. In an earlier study at these laboratories it was suggested that Verazide was recoverable from the serum of persons taking the drug. In the light of the present findings, this view is no longer held. It now appears that hydra-

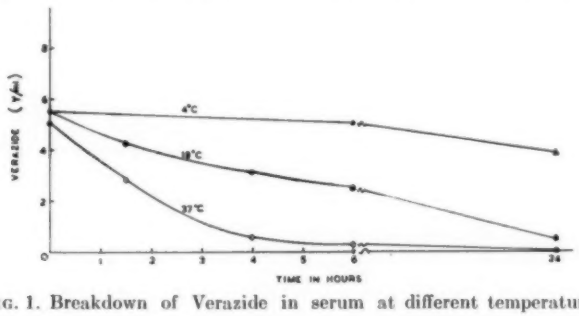

FIG. 1. Breakdown of Verazide in serum at different temperatures.

TABLE 4

PRODUCTS OF THE BREAKDOWN OF VERAZIDE

Experiment	Initial Verazide	After 12 Hours at 37°C. in Plasma			Verazide Accounted for	
		Residual Verazide	Isoniazid	*Iso*nicotinic Acid		
	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	*per cent*
1	39.5 (40.0*)	9.0 (8.4*)	9.9	6.0	43.5	110.0
2	37.5	4.0	11.2	4.0	36.6	97.5
3	34.0	4.0	11.0	3.5	35.0	103.0
4	39.5	5.0	11.0	3.5	36.0	91.2
Summary of Verazide breakdown from Experiments 1–4	37.6 100%	5.5 14.6%	22.5 59.8%	9.8 26.1%	37.8	100.5%

* Estimated by radioactive count. Radioactive Verazide was prepared from isoniazid labeled R-$C^{14}O$ NH NH_2, the specific activity of the isoniazid being 2 mc/mM. Radioactive counts were done, using a Phillips counter previously calibrated with the radioactive isoniazid.

TABLE 5

PLASMA CONCENTRATIONS OF HYDRAZONE AND ISONIAZID

Dose of Hydrazone: 10 Mg. per Kg. of Body Weight

Drug	Subject	Plasma Concentration (γ per Ml.) After Ingestion			
		2 hours		4 hours	
		Hydrazone	Isoniazid	Hydrazone	Isoniazid
Verazide	L. W.	0	3.0	0	2.8
	G. V.	0	1.3	0	0.8
	S. D. R.	0	2.0	0	0.8
Phtivazid	L. W.	0	1.2	0	1.9
	G. V.	0	0.5	0	1.0
	S. D. R.	0	<0.5	0	0.6
Nupasal-213 or Salizid	L. W.	0	1.2	0	1.1
	G. V.	0	0	0	0
	S. D. R.	0	0.8	0	0.5

In the estimation of isoniazid (6), no distinction was drawn between free and acetylated drug.

zones of isoniazid are quite unstable in neutral protein containing fluids such as blood serum and tissue homogenates and that their half-life is approximately two to four hours at 37°C. (table 3).

In this study, Verazide, Phtivazid, and Nupasal-213 (or Salizid) were included and all showed much the same rate of disappearance in blood and serum. Hydronsan®, sodium glucuronate *iso*nicotinyl hydrazone,[2] was examined and the preliminary findings indicate that it is also unstable in serum. These latter findings extend those of Colwell and Hess (12) who found that D-glucuronolactone *iso*nicotinyl hydrazone (Gatalone®) was unstable in serum and plasma. Furthermore, after oral administration of each of the three aromatic aldehyde hydrazones, none were detected in the plasma (table 5).

In a more detailed study of Verazide, its disappearance could be accounted for by the appearance of isoniazid and *iso*nicotinic acid (table 4). Hence, it would appear that the chemotherapeutic activity of Verazide (and the other hydrazones tested) is largely, if not entirely, due to the release *in vivo* of isoniazid. This view confirms similar conclusions reached on clinical grounds (1, 3).

The nature of the breakdown of the hydrazones has not been sufficiently investigated for comment at length. The reaction seems to be initially hydrolytic in nature and appears to be dependent on the presence of protein (table 2). While the activity is not affected by the heat treatment described (table 2) or the animal species (table 1), it is temperature sensitive (figure 1).

Finally, in regard to low toxicity of these hydrazones as found by animal experiment, this can be accounted for in the case of the aromatic aldehyde hydrazones by their low solubility and, hence, slow release of isoniazid.

SUMMARY

The stability of three hydrazones of isoniazid, which have been investigated clinically, has now been studied in various biologic materials. Particular attention has been given to the stability of Verazide in blood and serum.

[2] Kindly supplied by the Chugai Pharmaceutical Co., Tokyo, Japan.

The results show that the hydrazones, Verazide, Phtivazid®, Nupasal-213® (or Salizid®) are broken down rapidly in serum, whole blood, and tissue homogenates. The breakdown is probably hydrolytic in nature, protein dependent, and yields isoniazid and some *iso*nicotinic acid. It is suggested that these hydrazones owe their *in vivo* activity to the liberation of chemotherapeutically active isoniazid.

Sumario

Quimioterapia de la Tuberculosis: IV. Estabilidad de las Hidrazonas de la Isoniacida en los Humores Biológicos

La estabilidad de tres hidrazonas de la isoniacida, que han sido investigadas clínicamente, ha sido ya estudiada en varios productos biológicos, dedicándose atención en particular a la estabilidad de la Veracida en la sangre y el suero sanguíneo.

Los resultados revelan que dichas hidrazonas, la veracida, la Ftivacida® y la Nupasal-213® (o Salicida®), se desintegran rápidamente en el suero, la sangre integra y los homogeneatos de tejidos. La desintegración es probablemente de naturaleza hidrolítica, dependiente de la proteína, y rinde isoniacida y algún ácido *iso*nicotínico. Se sugiere que estas hidrazonas deben su actividad *in vivo* al desprendimiento de isoniacida quimioterapéuticamente activa.

Resume

Chimiothérapie de la tuberculose: IV. Stabilité des hydrazones de l'isoniazide dans les liquides biologiques

Après investigation clinique, la stabilité de trois hydrazones de l'isoniazide a maintenant été étudiée dans diverses substances biologiques. La stabilité de la Vérazide dans le sang et le sérum a été l'objet d'une attention particulière.

Les résultats montrent que les hydrazones, la Vérazide, la Phtivazide® et le Nupasal-213® (ou Salizid®) sont rapidement dégradées dans le sérum, le sang total et les homogénats tissulaires. La dégradation est probablement de nature hydrolytique, sous la dépendance de la protéine et a pour résultat la production d'isoniazide et d'une certaine quantité d'acide *iso*nicotinique. L'auteur avance que ces hydrazones doivent leur activité *in vivo* à la libération d'isoniazide active du point de vue chimiothérapique.

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MORPHOLOGY OF HEALING PROCESSES IN CHEMOTHERAPY[1, 2, 3]

Translation of a Soviet Review

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In recent times interest has increased in the analysis of the morphologic reactions of patients with tuberculosis under the influence of chemotherapeutic preparations. Most investigators believe that at the basis of the chemotherapeutic action of these preparations lies a bacteriostatic effect.

Berblinger (1) indicates that streptomycin, PAS, amithiozone, and isoniazid act bacteriostatically on the microbes. As a consequence of the restraint of growth of *Mycobacterium tuberculosis* under the influence of antimicrobial drugs, the tissues are only slightly damaged, as the result of which the natural defense mechanisms of the organisms are mobilized. He attributes great importance in this regard to the reticuloendothelial system.

There are indications that streptomycin elevates the activity of diamine-oxidase, which is very important in the metabolism of *Mycobacterium tuberculosis*. In a review devoted to the changes of tuberculous foci following chemotherapy (2), it is emphasized that the effect of antimicrobials, for instance streptomycin, is antibacterial and not related to effects on the tissues.

On the basis of a comparison of the different antimicrobials on *Mycobacterium tuberculosis*, certain investigators (3, 4) believe that isoniazid possesses greater bactericidal action than streptomycin. Under the influence of isoniazid, the tubercle bacilli are killed both inside and outside the cells. Experimental studies confirm the bactericidal action of streptomycin and isoniazid *in vitro* in both human and bovine types of *Mycobacterium tuberculosis* during reproduction of the microorganisms. Middlebrook and Yegian (5) and Schaefer (6) emphasize that in pulmonary foci of tuberculosis the mycobacteria are found in great numbers extracellularly, especially in open cavities (7). For patients with such changes, streptomycin and PAS may be effective. Liebegott (8) indicates that antimicrobials (for instance isoniazid), in addition to a restraining influence on the reproduction of the *Mycobacterium tuberculosis*, also exert a cytotoxic effect on the bacilli.

Studies devoted to the investigation of the morphologic reactions in tuberculosis under the influence of various antimicrobials introduced into practical use in the recent past may be divided into two basic groups.

On the basis of certain studies (the least numerous), it is confirmed that the healing of tuberculous lesions under the influence of antimicrobial drugs does not differ, in principle, from spontaneous healing. Some investigators believe that the quick and firm development of fibrosis in tuberculous foci under the influence of the antimicrobial preparations is related to the swift disappearance of the bacilli. This is especially well demonstrated in the healing of the fresh tuberculous foci. This view is upheld by various authors (9–13). They believe that no unusual changes in tuberculous foci can be noticed under the influence of streptomycin. Resorption of the perifocal reaction, closure of the cavity, and encapsulation of the caseous foci may also occur without the use of antimicrobials. The latter only increase the frequency of the favorable progress of processes. In acting upon the *Mycobacterium tuberculosis* and destroying it, the various antimicrobials act in the same way and through the same immunobiologic reactions of the organism which modify the protein structures of the plasma (14).

A much more numerous group of writers suggest that, while preserving the basic regular dynamics of tuberculous inflammation, antimicrobial drugs introduce many new features

[1] From the Department of Pathomorphology of the Central Institute of Tuberculosis, Academy of Medical Sciences, U.S.S.R.

[2] From Contemporary Problems of Tuberculosis, 1955, *6*, 308.

[3] Translation of this paper has been furnished by the National Institutes of Health, Public Health Service, as part of the Russian Scientific Translation Program.

into the morphologic reactions in the healing of tuberculous lesions. Thus, Canetti (15) alludes to new (in principle) cellular reactions under the influence, chiefly, of isoniazid. He describes the appearance in the lungs of tuberculous patients of unique epithelioid and plasma cells which create the morphologic basis for a pneumonia of a special form, which develops with the use of antimicrobials. Poppe de Figueiredo and De Paolo (16) describe the appearance of giant cells during therapy with isoniazid, and also remark on the appearance of a marked hyperemia of the interalveolar septa during treatment with this preparation.

Auerbach (17) affirms that, in the chemotherapy of tuberculosis, a marked thinning of the fibrous capsule occurs around the necrotic foci. One of the unique features to which he points is the diminution of fibrosis of the lungs and the development of emphysema.

It should be noted that the majority of the articles relate to the changes in the lungs of man and the experimental animal with various forms of tuberculosis. The changes in the other organs are described only by occasional writers (in the larynx, kidneys, et cetera).

All investigators describe separate stages in the modification of the tuberculous inflammation under the effects of the antimicrobials. Thus, all of the writers, without exception, dwell primarily on the changes of the perifocal reaction under the influence of antimicrobials. The perifocal reaction usually, as Auerbach describes (17), is characterized by the presence in the alveoli of edema, erythrocytes, and alveolar phagocytes. Sometimes it takes the form of tuberculous pneumonia (first of the "red," and then of the "gray" types) or converts to gelatinous pneumonia. This perifocal reaction, under the influence of antimicrobials, is quickly reduced. In this, the edema and the cellular elements are resorbed and the remnants of cells near the inflammatory focus are replaced by connective issue. The perifocal reaction disappears, as is apparent even under clinical observation. The pathologic anatomy of this has been clearly described and demonstrated. In consequence of the disappearance of the perifocal reaction, a unique histologic picture is established of specific changes when the encapsulated foci in the lungs are changed almost to the appearance of unaffected pulmonary tissues (2, 18).

In certain cases treatment leads not to resorption but to organization of the perifocal reaction of inflammation (15). As the result of this, interstitial sclerosis develops, terminating in generalized carnification.

Much attention has been given to the morphologic changes in the tubercles. These tubercles, according to the description of Richter (11), during treatment with isoniazid take on the form of epithelioid-cell foci distributed among unchanged pulmonary parenchyma. Around the peripheral tubercles a clearly defined wall of lymphocytes is noticed. Cannetti (15) noted a significant degree of fibrosis occurring in the tubercles, with eventual hyalinization and a pronounced diminution of their size.

In connection with the use of the new antimicrobial preparations, the morphologic reactions in tubercles are not limited solely to fibrosis with hyalinization, as indicated above, but a number of qualitatively new signs appear. Thus, Liebegott (8) describes the appearance of a large number of vessels in fresh tuberculous lesions in the lungs during treatment with isoniazid. In the Langhans' cells significant changes occur in the size and number of nuclei. In the late stages of tubercle formation, one may observe fragmentation of the tubercles with the appearance of nonspecific granulation tissue rich in capillaries. Finally, as a trace of the tubercles, there remains only a hyalinized connective-tissue cicatrix in which isolated capillaries may be seen.

In large caseous foci, according to the observations of a number of writers (1, 11, 15, 18–20), the influence of the antimicrobials is less clearly expressed than in the tubercles. However, the writers point to the diminution in the thickness of the capsule around the caseous foci as a phenomenon combined with the resorption of the perifocal reactions (18, 21). Along with this it is noted (21) that, in combining treatment with streptomycin and isoniazid, an increase takes place in the vascularization of foci and resorption of caseous material, and a diminution in the number of epithelioid cells. Poppe de Figueiredo and De Paolo (16) note, in addition to the resorption of the specific foci following the use of isoniazid, the appearance of swift carnification of widespread areas of alveoli filled with macrophages. However, the majority of writers believe that the principal effect of treatment with isoniazid-streptomycin is the development of processes of

resorption of tuberculous foci with minimal fibrosis. In this, the caseous material is resorbed. Around old caseous foci and in the center of them, there is some hemorrhage (20, 21). Not only dilatation of the blood vessels in the granulation tissue but also new formations of them may be observed. The quick resorption of perifocal inflammation and the encapsulation of caseous material lead to the appearance in the lungs of isolated caseous foci of the tuberculous type (19).

The changes described are especially well expressed in fresh tuberculous foci. In the larger foci of specific pneumonia, along with processes of resorption and the development of fibrosis, formations of lymphoid follicles are observed; in this process, the epithelioid cells are again converted to macrophages, large numbers of giant cells appear, the alveolar epithelium is regenerated and assumes a cuboidal shape, and regeneration of the bronchial thelium occurs (15, 21). It is possible to see all of the transformations from large round macrophages to actual epithelioid cells. The giant cells are distinguished by considerable polymorphism, which will be discussed further.

The described metaplasias of cells are encountered not only in the region of the developing sclerotic changes, but also at the periphery of large caseous foci. Sometimes they are discovered to the side of the caseous foci and present the picture of unusual alveoli filled with epithelioid or giant cells (15). All of the transitions from the described transformations of the cells to fibroblasts and fibrocytes are observed in the complete cicatrization of the foci. Sometimes, in progressive processes during the period of treatment, a secondary caseation of the areas described ensues. It presents a unique picture and is characterized by necrosis on the background of fibrosis in the absence of an exudative phase.

The swift development of the processes of resorption and healing of the tuberculous foci with epithelization of them can be observed in patients with tuberculosis of the larynx (13).

Great attention has been given to changes occurring in the walls of cavities under the influence of treatment. It is well known that healing of cavities under ordinary conditions may occur in three ways: (*1*) closure of a draining bronchus, with consolidation and calcification of the enclosed cavity; (*2*) formation of connective-tissue cicatrices at the site of the cavities, and (*3*) fibrosis of the wall of the cavity with subsequent epithelization of the residual cavity from the draining bronchus—open healing of the cavity (22).

In the use of the various chemotherapeutic preparations, the investigators note a number of peculiarities in the process of healing of the cavity. First, the resorption and swift organization of the perifocal reaction leads to a reduction in the thickness of its wall (7, 17). Then, in the wall, processes of fibrosis and development of nonspecific granulation tissue supervene. The swift development of fibrosis is characteristic (15, 22, 23).

Caseation in the wall is replaced by nonspecific granulation tissue, in which accumulations of epithelioid and large giant cells may be found. (1, 16, 22). Certain writers believe that it is more characteristic of the healing of cavities, under the influence of antimicrobials, to find fibrosis of its wall and development of connective tissue cicatrices in its place (24). Much attention, particularly recently, has been given to the so-called open healing of cavities (1, 8, 22, 25–30). In this, the formation of cysts with thin, smooth walls at the site of the cavities is especially characteristic of the effects of antimicrobials, especially isoniazid (7, 17, 22, 28, 31). Epithelization of the wall of such a residual cavity is not obligatory. Some writers (22) believe that epithelization of the cavity proceeds from the draining bronchus, while others (16) think that a special metaplasia of the connective tissue cells takes place.

Not infrequently, healing of cavities may be observed in some patients in a different way. In the so-called open healing it is not always possible to observe immediate clearing of all cavities. Often, along with complete clearing and fibrosis of some parts, caseous material may be seen in other parts. Unusual healing of cavities of the open type may also be observed with simultaneous development of caseous foci and epithelization of one region of the wall (22). Canetti (15) also regards the existence of three types of healing of cavities: the full cavity, filled with caseous masses; the clearing cavity; and finally the cystic type. In the open healing of cavities, during their clearing, healing and epithelization of the bronchus occurs. In closed healing the bronchus becomes fibrotic, and its lumen becomes obstructed. Auerbach (17), Canetti (15), and others believe that it is due to chemotherapy that one

observes transformation of the cavernous space into a cyst without obliteration of the bronchus.

It is necessary to remark that several writers, studying the morphologic reactions in the chemotherapy of tuberculosis, do not distinguish the peculiarities of the action of each antimicrobial separately (1, 17). Other writers, such as the Soviet morphologists, V. I. Puzik and F. I. Chumakov,[4] distinguish carefully between the morphologic reactions obtained upon the administration of streptomycin, on the one hand, and isoniazid and its derivatives, on the other. Particular attention is given to processes of resorption of caseation, best shown with the use of isoniazid. The authors relate these phenomena to the appearance of unusual giant cells with vacuoles and various cytoplasmic inclusions (15, 32). Thus, Canetti (15) describes the appearance of cells of giant dimensions of the foreign-body type, surpassing many times, with respect to size, the ordinary giant cells of the Langhans' type. Liebegott (8) describes in these cells large numbers of hyperchromic nuclei; and, in the cytoplasm he describes vacuoles, phagocytized cellular fragments, and leukocytes. Poppe de Figueiredo and De Paolo (16) describe the appearance of foamy giant cells with fatty inclusions. Denst (32) and Pagel and Simmonds (22) describe similar cells.

In addition to the described phenomena of the resorption of specific foci and the related appearance of large giant cells, many writers point to the presence, during treatment with isoniazid, of hyperemia in both the tuberculous foci and the regions around the cavities, as well as of hyperplasia and proliferation of the epithelium (16).

Many investigators have given much attention to proliferative processes in the use of chemotherapy. They note not only the appearance of unusual forms of giant cells but also the proliferation of distinct, large, often vacuolated, epithelioid cells (15, 16, 20). They describe transformation of mesenchymal cells into epithelioid cellular elements which take an active part in the processes of healing of the tuberculous foci and of the cavities. Especially noticeable are the processes of proliferation of cells and the transformation of the epithelioid elements into monocytes during treatment with isoniazid. In treatment with streptomycin alone, or with streptomycin–PAS, there is much less evidence of this (20). In treatment with streptomycin alone, there is a predominance of processes not of resorption but of fibrosis (20, 21).

In the problem of the study of the combined use of antimicrobials, certain writers concentrate on the question of their antagonistic action (33). As the result of experiments which have been performed, they have reached the conclusion that the combination of therapeutically active doses of isoniazid with small amounts of streptomycin does not result in antagonism.

In the experiments performed, the investigators have used not only experimental studies and examination of the sectioned material, but also the material from major operative procedures as well as from biopsies.

In the recent past, morphologists have begun to study the reactions arising with the administration of endocrine preparations (corticotropin, cortisone, and others) along with the chemotherapeutic agents. This question, however, is as yet insufficiently analyzed in the literature. The introduction into practice of endocrine preparations once again points to the importance of the status and condition of the host in the problem of the treatment of tuberculosis.

[4] V. I. Puzik: Pathologico-morphologic characteristics of processes of healing of cavernous tuberculosis under treatment with antibiotics and chemopreparations: first collection in *Surgical Methods of Treatment in Tuberculosis*, Medgiz., 1954, p. 100.

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ALVEOLAR CELL CARCINOMA[1]

A Review with a Report of Four Cases

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Introduction

Alveolar cell carcinoma was first described by Malassez (1) in 1876 and, in 1903, Musser (2) reported the first case of diffuse alveolar cell carcinoma. In 1907, Helley (3) described a multiple nodular bilateral tumor; since then more than 200 cases have been reported in the literature.

Various names are given to this type of carcinoma, such as: adenoma-like tumor, malignant adenomatosis, multicentric-cell tumor, bronchiolar carcinoma, pulmonary adenomatosis, et cetera; and Liebow (4), in 1952, collected more than 36 of such cases. The etiology of this disease is still obscure, and different views are expressed by different writers.

According to some writers, various physical and chemical irritants can be responsible for development of alveolar cell carcinoma, whereas others say that chronic irritation and heredity are responsible. Furthermore, it has been pointed out that there is a morphologic resemblance between alveolar cell carcinoma in man and jagziekte, an infectious disease of viral origin in sheep; but experimental transmission of this disease from sheep to man has failed so far.

Symptoms and Signs

In the present series of 4 cases were one male and 3 female patients; one was forty-four and the others were between fifty and sixty years of age. In all 4 patients the onset of disease was insidious and all had symptoms. The duration of symptoms in the 4 patients varied: in one case, the symptoms were of six months' duration prior to admission to this hospital; in 2 cases the symptoms lasted for twenty-four months; and one patient had a cough "for years." Cough was the main symptom in all 4 cases. Two patients had thin watery sputum; one had been coughing up to 30 to 45 ml. of sputum; another, 180 to 240 ml. in twenty-four hours; the third, a little; and the fourth had no sputum. Dyspnea was marked in one case; the 3 other patients were breathless on exertion. Pain was a late symptom in 2 cases, and loss of weight and energy was present in 2 cases. Cyanosis was marked in the terminal stages of the disease in 3 cases, and none of the patients coughed up blood at any time during the course of the disease. Three patients were afebrile, and one had a temperature ranging between 99° and 100° F.

The physical signs were not of great help; two patients had impaired percussion notes with weak breath sounds in the lower part of the right lung; the third patient had crepitations in the lower part of both lungs; and in the fourth there were no signs.

Roentgenographic Findings

There are no pathognomonic roentgenographic appearances of alveolar cell carcinoma, and the findings vary from case to case, mimicking any form of bronchial or pulmonary disease. The following findings are usually described:

(*1*) Unilateral or bilateral nodular densities from the miliary size, 2 mm., to 2 cm. in diameter, occupying an area of a lobe, one lung and, more commonly, both lungs
(*2*) A solitary round shadow, varying in size, usually situated peripherally
(*3*) Scattered densities, ill-defined in outline, resembling those of bronchopneumonia
(*4*) Homogenous areas of pneumonic consolidation occupying a lobe or the whole lung
(*5*) Pleural effusion
(*6*) A combination of the various forms situated in either lung or in both.

In the present series, in the first case, the chest films showed a large round density in the right lower zone with collapse of some segments of the right lower lobe and a small pleural effusion; the left hemithorax appeared to be normal (figure 1). However, subsequent chest films obtained three months later showed nodular densities in the remaining right lung and in the left lung also (figure 2). Whether these nodular densities in the

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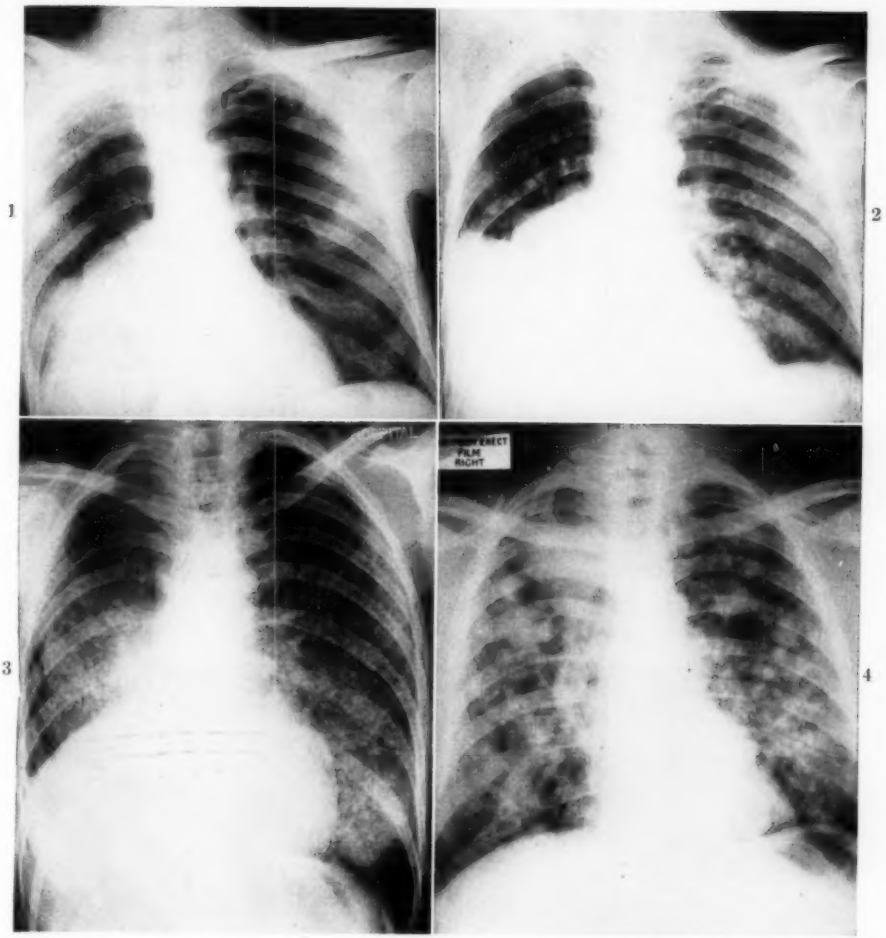

FIG. 1. (Upper left). The postero-anterior chest film shows a rounded density situated in the right lower zone. The left lung is normal.

FIG. 2 (Upper right). The postero-anterior film of the chest taken three months later shows that the density has increased in size. There is also a fluid level in the right base *and* there are many scattered densities in the remainder of the right lung and in the left lung also.

FIG. 3 (Lower left). Showing miliary densities scattered throughout both lungs. There is a collapse of part or of the whole right lower lobe.

FIG 4 (Lower right). Showing irregular nodular opacities scattered throughout both lungs.

left lung were present but undetectable when the disease on the right side was discovered, or whether the densities represent a spread from the right lung, is an open question. The other three chest films showed bilateral nodular densities occupying the fields of both lungs. Tuberculosis was the diagnosis suggested on the basis of the appearance of chest films in 2 cases. One patient was treated for pulmonary tuberculosis, later sarcoidosis, and the diagnosis of alveolar cell carcinoma was finally made post mortem (figures 3, 4, and 5).

Pathologic Findings

Macroscopic findings: Alveolar cell carcinoma usually begins in the periphery of the lung and is seen as a nodule from 2 mm. to about 10 cm. in diameter; it may be solitary, usually large, or there may be many scattered over areas of one lobe, one lung, or of both lungs. These nodules may coalesce and resemble a stage of gray hepatization of pneumonia (figure 6); or, if they are of miliary size, they resemble caseating tubercles (figure 7). A central area of larger nodules may become necrotic and a cavity may form; and on cut sections the surface looks gray and mucus exudes from it. There is an absence of an intrinsic tumor in the bronchial tree, and the bronchi are usually filled with thin and watery fluid. The weight of the lungs may be increased, as it was in the first case described below in which the weight of the right lung was 2,210 gm. The intervening lung parenchyma not affected by neoplastic changes showed various degrees of pneumonia, fibrosis, and compensatory emphysema. The pleural cavity contained a small amount of serous fluid in one case and adhesions were also found in 3 cases.

Microscopic findings: The alveoli were lined with tall columnar cells, the cytoplasm of which was eosinophilic; the nuclei were round or oval and were situated basally. These cells showed papillary proliferation into alveolar spaces, and mitotic figures were found in 2 cases (figure 8). The secondary changes consisted of areas of pneumonia, necrosis, fibrosis, and of compensatory emphysema (figure 9).

The question whether alveolar cell carcinoma is of multicentric or unicentric origin is still unsettled. According to the writers who favor the unicentric origin, the alveolar cell carcinoma arises from one focus and then spreads by means of the blood vessels, lymphatics, and by bronchiolar dissemination, giving rise to new foci of growth. The argument for the multicentric origin is the recurrence of the new lesion in the contralateral lung after the resection of the tumor from one lung months or even years later. However, most authors agree that this tumor may spread by blood vessels or by lymphatics and air passages.

Storey and associates (5), in their series of 208 cases, found that in 54 per cent there was evi-

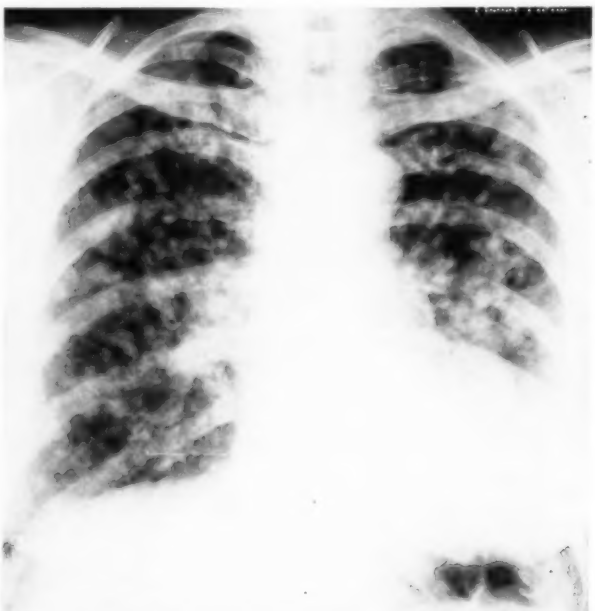

Fig. 5. Showing irregular nodular opacities scattered throughout both lungs.

dence of spread: in 38 per cent of this group, to mediastinal lymph nodes; and in about 16 per cent (15.6), spread was noted to distant organs such as liver, abdominal nodes, bones, adrenals, brain, and kidney, in this order.

In all 3 cases the microscopic examination showed secondary deposits in the regional and mediastinal lymph nodes, and in 2 patients vascular invasion was also found.

In the first case described below, the patient developed a right hemiplegia with aphasia about eight hours after bronchograms were obtained. This patient died five months later with an abscess in the brain, but no neoplastic disease was found in the brain, although it was thought at this time that the symptoms had been due to metastatic tumor.

In the the third case, there was also a considerable invasion of the pulmonary vessels and lymphatics, and emboli were thus formed. The invasion of pericardium and myocardium by alveolar cells throughout the lymphatics was also seen (figure 10). Swann's (6) three criteria for a diagnosis of the alveolar cell carcinoma: proliferation of the alveolar cell with the tall columnar mucus-producing cells, absence of the intrinsic tumor in the bronchial tree, absence of an adenocarcinoma in other systems of the body, were fulfilled in all four cases—three at necropsy, and one on clinical examination.

Case Reports

Case 1. The patient, A. H., was a male, fifty-five years old, whose family history was unrevealing. He had had a partial gastrectomy five years previously; otherwise, he had never been seriously ill. The present illness had begun six months before admission to the hospital and consisted of a gradually increasing dyspnea, accompanied by a cough which was dry at the beginning and recently had become more productive. He also complained of general weakness, anorexia, and loss of fourteen pounds in weight during this time. The patient never coughed blood, but had had a slight chest pain, and his fingers had become clubbed in the three months before hospitalization. He had smoked ten to fifteen cigarettes daily since early youth.

On physical examination, the patient's general condition was fair and he looked rather dyspneic. He was not cyanotic, and had marked clubbing of the fingers. On examination of the chest, the percussion note was impaired and the breath sounds were suppressed over the right lower lobe. No abnormalities were found in the other systems on physical examination. Examination of the blood

Fig. 6 (Left). Photograph of the specimen shows a consolidated part of the lung consisting of the confluence of nodular masses.

Fig. 7 (Right). Photograph of the specimen (right lung) shows nodular small densities in the whole right lung with a tendency to confluence in the right middle zone.

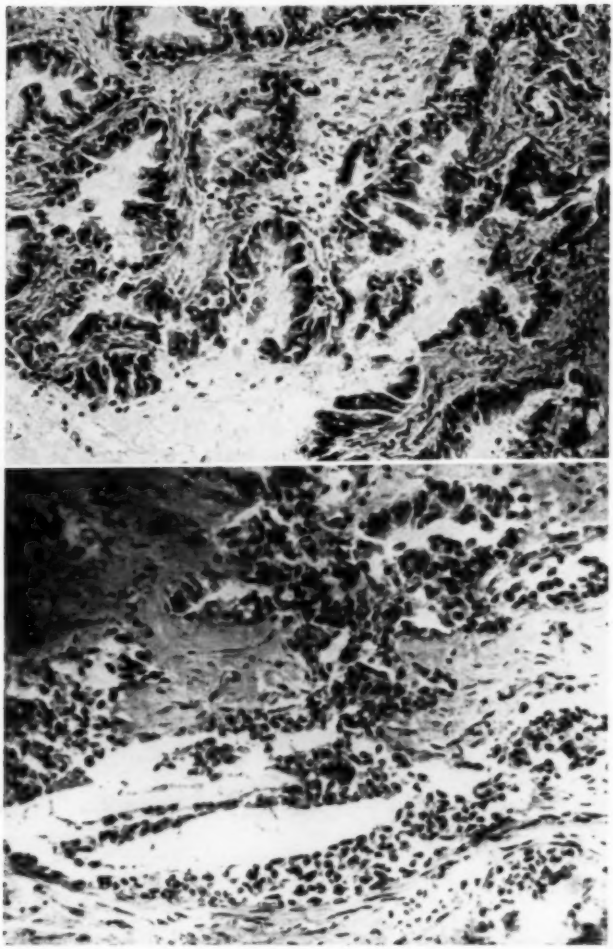

FIG. 8 (Upper). Photograph shows a proliferation of the tall columnar cells in the alveoli.
FIG. 9 (Lower). Photograph shows a proliferation of the tall columnar cells in the alveoli with a marked interstitial fibrosis.

and the urine revealed no abnormalities. Sputum cultures were negative for tubercle bacilli.

Chest films revealed a large round opacity in the right lower lung field lying posteriorly, with a collapse of the posterior segments of the lower lobe and with an interlobar effusion. The mediastinal lymph nodes seemed to be enlarged. The left lung appeared normal.

On bronchoscopy, the vocal cords, trachea, and carina were normal. Only the right bronchus was examined. There was an over-all and slight generalized narrowing of the right main bronchus. In the lower lobe bronchus, a large amount of rather watery mucus was noted. About 45 ml. of this were aspirated, and later samples were considerably blood-stained and contained flecks of some other material. There was no definite bronchoscopic evidence of a carcinoma.

Thin mucus with openly scattered cells was aspirated (the majority polymorphonuclear and alveolar), with only rare bronchial epithelia. Many pathologic cells were present. From evidence of anthracotic inclusions and nuclear multiplicity, these were almost certainly of alveolar histiocytic origin and represented a very extreme degree of metaphasia or possibly neoplasia such as alveolar cell carcinoma.

Bronchograms showed a blockage of the apical

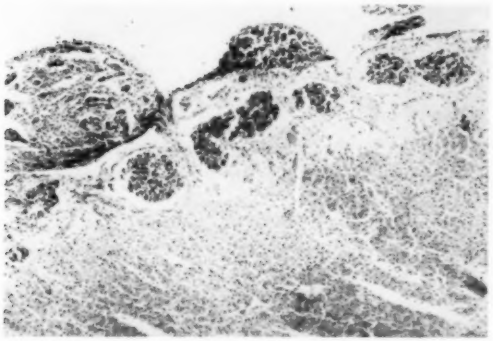

FIG. 10. Photograph shows the malignant cells invading pericardium and myocardium. Innumerable alveolar carcinoma cells in the pericardial lymphatics.

bronchus of the lower lobe; otherwise they were normal. Unfortunately, eight hours after bronchograms were obtained, the patient developed a right-sided hemiplegia with aphasia. This was attributed to metastases in the brain and, therefore, a course of prednisolone, with antimicrobials, was given.

During his stay in the hospital, dullness in the right lower zone, noted on admission, was steadily increased, and moist sounds were heard over areas of both lungs. The patient had a temperature ranging from 97° to 99°F. which went up to 100°F. once or twice weekly. The pulse rate was between 100 and 110 per minute. His dyspnea increased and he coughed thin and watery sputum, varying in amount. At the beginning it amounted to 15 to 45 ml.; then it increased to 180 to 240 ml. in twenty-four hours. The patient's appetite was fair, but he was losing weight and strength. The leukocyte count was between 27,000 and 30,000, and blood chemistry was normal. However, the chest film showed scattered densities in the left lung *three months* after admission to the hospital for the first time. His cerebral symptoms improved, but he never regained his speech. He died five months after admission.

Post-Mortem Examination

Macroscopic findings: The brain was removed and a gelatinous exudate was noted over the left hemisphere as well as an area of softening toward the center, with flattened convolutions. On opening the lateral ventricles, a thick purulent-like matter lined the floor on the left side, and on incision this opened into an associated abscess cavity within the left central hemisphere, containing similar material. There was some cystic dilatation of the left choroid plexus. Cerebrospinal fluid which escaped during removal of the brain was clear. No abnormalities were discovered in the medulla, pons, or cerebellum.

The trachea and main bronchi to both sides of the thorax were filled with thick frothy mucus, which, on the right, was chocolate colored, with associated hemorrhagic petechiae of the bronchial mucosa (right side only). The right lung was almost completely consolidated and nonadherent in a *dry space*, part of its middle lobe and part of the upper lobe still aerating; this lung on removal weighed 2,210 gm., and on incision released thick glutinous mucus from the incised surface and bronchi. A consolidation of caseous-looking material was present throughout the whole lower lobe and in the bulk of the upper and middle lobes; this material was Ziehl Neelsen negative. There was a large mucus-containing "abscess" in the right cardiac segment which was very exceptional. The left lung was fully aerated and fixed by adhesions at the apex, posterior margin, and diaphragmatic surface, although the space was dry. Palpation of this lung revealed nodular masses which on incision resembled the disease in the right lung.

No specific tumor could be demonstrated within the lung, but a condition of diffuse carcinomatosis was the macroscopic diagnosis. Mediastinal lymph nodes which were incised, although not much enlarged, were indicative of tumor invasion. Examination of other organs revealed no abnormalities.

Microscopic findings: The lung sections showed widely dispersed alveolar cell carcinoma with secondary changes comprising mainly areas of compensatory emphysema, pneumonia, and of mucus-dilated alveoli.

All of the suspect lymph nodes examined from the hilar and mediastinal region were completely replaced by carcinoma; vascular invasion was also present. Metastasis was not present in the various bone sections examined.

The lesion in the brain was nonhemorrhagic cerebral softening with no tumor metastases. Sections of other organs showed no abnormalities.

Case 2. The patient, T. C., a 54-year-old male, was admitted to the hospital in January, 1955, complaining of a cough and sputum production. He had been well, except for a winter cough, until 1953. Since that time his cough had become troublesome and productive of watery sputum (but never blood-stained) two or three times in

twenty-four hours. In addition, he had developed increasing dyspnea.

On physical examination, the patient was plump and healthy looking, although dyspneic on slight exertion. Except for some crepitations at both bases of the lungs, the examination showed no significant abnormality in respiratory and/or other systems.

Examination of the blood and urine revealed no abnormalities. His sputum was positive for acid-fast bacilli on direct examination, and his reaction to a tuberculin test (Mantoux, 1:1,000) was positive. An intravenous pyelogram was normal; the albumin-globulin ratio was 1.5:1. The serum alkaline phosphatase was 4.5 units; total serum proteins, 6.0 gm. per 100 ml.; albumin, 3.6 gm. per 100 ml.; and globulin 2.4 gm. per 100 ml.

A chest roentgenogram (he was said to have had a normal roentgenogram five years previously) showed large nodular diffuse fluffy opacities scattered throughout both lungs. Roentgenograms of the hands and feet showed no bony abnormalities. A bronchoscopy was not performed.

In June, because of the lack of response to antituberculous therapy, the possibility of sarcoidosis was considered, and 100 units of corticotropin were given daily in addition to streptomycin and isoniazid. At first there was some symptomatic improvement, but this was not maintained.

The patient became steadily more dyspneic and continued to produce profuse watery sputum. There was no wasting, and no significant change was noted in the appearance of his roentgenogram; but he went steadily downhill and died on August 8, 1955, seven months after he was first seen.

Post-Mortem Examination

Macroscopic findings: The trachea and bronchi contained some brown, "soupy" pus which extended far down into the lung. Pleural adhesions were present only in the apex of the right lung; otherwise the lung was smooth with no fluid in the pleural cavity. Both upper lobes, the right middle lobes, and the apices of both lower lobes of the lungs showed deep fissuring on their surface, outlining chunks of lung 1 inch, 2 inches, and 3 inches in longest diameter. Palpation of these parts of the lung outlined multiple rubbery nodules in the lung substance. When the lungs were cut, these areas showed up as gray, dry patches, criss-crossed by strands of fibrous tissue, much more evident to the touch than to the sight. The right lower lobe showed widespread consolidation of a different type, much more moist and yellow. Many medium and small branches of the pulmonary artery were plugged by thrombus which was adherent to the vessel wall. There was one large lymph node below the bifurcation of the trachea around the hilar of the lungs, and in the supraclavicular fossae there were small shotty nodes. No serious abnormalities were discovered in the other organs.

Microscopic findings: Sections showed alveolar cell carcinoma, infiltrating both lungs. There was considerable fibrosis, indicating that the growth had produced it as it had spread; the solid areas in the lower lobes were infarcted areas in which there was considerable polymorphonuclear leukocytic infiltration. Six lymph nodes from the hilar, paratracheal groups all contained secondary deposits. No serious abnormalities were revealed in the other organs.

Case 3. The patient, M. E., was a female, forty-three years old, whose family history was unrevealing. She had never had any serious illness. The patient was examined during a mass roentgenographic survey because she had had a cough for some years; this appeared worse at night but was unproductive and she had never coughed blood. She had a slight pain in the right chest and was dyspneic on exertion. Her weight had remained steady and her appetite was normal.

On physical examination, the patient looked ill. There was a moderately diminished movement of both sides of the chest, particularly over the right lower lobe, which was dull to percussion; the breath sounds were suppressed. No abnormalities were found in the other systems on physical examination. Examination of the blood and the urine revealed no abnormalities. The erythrocyte sedimentation rate was 23 mm. per hour; 53 mm. in two hours (Westergren). The cerebrospinal fluid findings were normal.

A chest roentgenogram obtained on admission to this hospital showed diffuse mottling ("snowstorm") throughout both lungs and some collapse of the right lower lobe.

On bronchoscopy, the bronchial tree was normal down to the middle-lobe spur, which was red and edematous. The middle opening contained pus. The right lower-lobe stem was converted to an antero-posterior slit by pressure from without, but the bronchoscope could be passed through this slit and no intraluminal lesion was seen other than a reddened swollen mucosa through the right lower lobe. The basal segmental openings were all blocked by the swollen mucosa and/or pressure from without. A bronchial biopsy and aspirate were performed. A number of narrow strips of bronchial mucosa showed some subepithelial cellular infiltration (polymorphonuclears, lymphocytes, and macrophages). Another strip consisted of rather abnormal epithelial cells, but without any stroma. There was no definite evidence of carcinoma.

A bronchial aspirate revealed no malignant cells. A lung biopsy was performed on April 23, 1958. There were no pleural adhesions. The lung was pale, gray, and indurated. It was finely granular, with minute cysts between the granules. A portion of the tip of the middle lobe was removed for section. The cut surface was gray, mushy, and avascular. The histologic report was alveolar cell carcinoma.

After operation the patient complained of a headache and she was increasingly dyspneic. Her pulse went up to 120 per minute and remained at

this level until her death. She died suddenly about two months after admission to the hospital.

Post-Mortem Examination

Macroscopic findings: The right lung was loosely bound down to the chest wall by fibrinous adhesions, and there was a small quantity (70 ml.) of turbid yellow fluid posteriorly. The lung was voluminous and irregularly nodular with collapse of the lower and middle lobes. There was a recent white pleural scar anteriorly over the middle lobe from which biopsy material had been taken. The lower lobe on section showed "oak leaf" areas of opaque yellow-white necrosis, and elsewhere the necrotic tumor had a light pinkish papillary appearance. There was no obvious abnormality of the basal bronchi. In the upper lobe there were small whitish nodules (average 0.4 cm. in diameter) in the lung substance, most conspicuous toward the hilum. The bronchi contained turbid watery mucoid material which on microscopic examination was seen to contain bronchial epithelium and numerous mixed microorganisms. The hilar tracheobronchial and paratracheal lymph nodes were small and anthracotic. All of the lobes of the left lung appeared similar to the right upper lobe (left lung reserved for further examination after fixation). There was a minimum of fluid in the left pleural cavity. No abnormalities were found in the other systems on examination.

Microscopic findings: The lung was the seat of alveolar cell carcinoma; there was heavy invasion of the lymph nodes where the tumor structure was less regular than in the lung primary. There was considerable invasion of the epicardial lymphatics of the heart, with occasional vascular invasion; a lymphatic spread into the myocardium occurred. (The surface fibrinous pericarditis was in fact due to the tumor plus the associated lymphocytic infiltration.) At least one small branch coronary contained an ante-mortem thrombus, and one fragment of intracardiac thrombus was also present ante mortem and on its surface lay a group of four tumor cells.

Case 4. The patient, C. B., was a female, sixty years old, who was admitted to the hospital with an irritating cough which had lasted for four months. She had never coughed blood. She had been dyspneic for the past six months, with lassitude and loss of energy for two years; she had lost 14 pounds in weight during the past year. She was a nonsmoker.

On physical examination, the chest was clinically clear; there was no evidence of neoplasm in other systems on physical examination. Examination of the blood and the urine revealed no abnormalities. Abnormal cells were present in small numbers in the sputum which were almost certainly neoplastic in origin; their morphology suggested a nonbronchogenic source. Culture of the sputum was negative for acid-fast bacilli.

Chest roentgenograms showed bilateral nodular densities occupying all fields of both lungs; a roentgenogram of the bones was normal. An intravenous pyelogram disclosed no abnormalities.

The findings on bronchoscopy were as follows:

> The whole bronchial tree contained a moderate amount of thin mucoid secretion. Cords, trachea, carina and right bronchial tree appeared otherwise normal. Left main and left upper normal. The basal bronchi on the left side were narrowed and the mucosa of the carinae between them was thickened and bled easily. A biopsy was taken from here. Two trap specimens of bronchial aspirate were obtained. Conclusion: Mucosal infiltration left lower lobe probably malignant. Nature of bronchial secretion suggests pulmonary adenomatosis.

The bronchial biopsy performed on July 22, 1958, revealed that the bronchial mucosa was invaded by the carcinoma cells and that there was also a considerable involvement of the lymphatics.

One bronchial aspirate contained a lot of blood, throughout which were scattered flecks of mucus filled with carcinoma cells. A second aspirate contained mainly bronchial epithelium and mucus against which background there were more rare flecks with carcinoma cells. All of these cells were unlike the common type found in bronchial carcinoma. Patchy distribution rather suggested endo-alveolar origin.

At thoracotomy no free fluid was found in the pleural cavity. The surface of all three lobes was studded with grayish white nodules one-half to three-fourth inches in diameter. Numerous nodules could also be felt in the depth of the lung. The parietal pleura was free from nodules. The histologic report showed an alveolar cell carcinoma.

The patient is now improving. Her appetite is better and she is gaining weight.

Comment

Clinically and roentgenographically, alveolar cell carcinoma may resemble any lung disease such as a specific and nonspecific infection, primary or secondary neoplasm. Diagnosis can usually be made from the history, clinical findings, roentgenographic appearances, bronchoscopy, and cytology of the sputum and of the lung biopsy. The alveolar cell carcinoma is not uncommon and its incidence varies between 1.5 and 5 per cent of all types of pulmonary carcinoma. There have been 327 male and 30 female patients with carcinoma of the lung and 4 cases of alveolar cell carcinoma in this hospital during the last six years. It occurs usually between thirty and seventy years of age. The patients in the present series were between forty and sixty years.

While carcinoma of the bronchus occurs pre-

dominantly in male patients, there is a higher incidence of alveolar cell carcinoma in female patients. In Decker's (7) series of 155 patients, there were 92 male and 63 female patients; in Watson and Smith's (8) series of 33 cases, there were 25 male and 8 female patients; in this series, there were 3 female and one male patient. The onset of disease may be either insidious or abrupt, and the symptoms are those of any pulmonary disease. Cough is usually described as a main symptom, which is dry in the early stages, but later may be productive with a large quantity of clear, frothy, watery sputum, the amount varying greatly from case to case. This thin, watery, frothy sputum is the only pathognomonic symptom, and the amount may vary from ounces to gallons in twenty-four hours, and it occurs in later stages of the disease.

Fox (9) described a patient who in twenty-four hours coughed up between 2,839 and 3,789 ml.; Rey and Rubinstein (10) described cases in which patients coughed up about 1,500 ml. in twenty-four hours. The production of clear, watery sputum, however, does not occur in every case of alveolar cell carcinoma. Cough was a main symptom of all 4 patients in this series; 2 produced a thin, mucoid sputum, the third had a little, and the fourth had none. Dyspnea varied in degree and increased as the disease progressed; cyanosis appeared in the terminal stage. A small hemoptysis or blood-stained sputum occurred in about 30 per cent of the cases in the literature, but was absent in the present series; however, loss of weight and energy was marked in 2 cases. Fever is not a constant feature; it was present in one case, and was due to superimposed lung infection. Duration of symptoms from the time of admission to this hospital to the date of death of 3 patients was between two and seven months. Roentgenographic appearances are not characteristic and may mimic any pulmonary disease, such as pulmonary tuberculosis, sarcoidosis, atypical pneumonia, and so on. The bilateral nodular densities occupying all the fields of both lungs were present in 3 of 4 cases.

The physical signs in the present cases were not of clinical significance and usually depended upon the type, the localization, and the extent of the lung lesions, and varied greatly from case to case, usually resembling those of pneumonia.

Bronchoscopy was performed in 3 cases, and the findings included a reddened, edematous mucosa which bled easily on touch, watery secretion, and narrowing of the bronchi with no intrinsic tumor. Cytology of the bronchial aspirate was positive for malignant cells in 2 cases.

Sputum examination may reveal the malignant cells, as was found in one case; and lung biopsy by thoracotomy is a deciding factor in diagnosis in doubtful cases, as it was in 2 cases of the present series.

In this series of 4 cases the diagnosis of the first was made on examination of the bronchial aspirate. The second patient, after excluding the presence of metastasis, was treated as a case of pulmonary tuberculosis (later as a case of sarcoidosis), and the diagnosis of alveolar cell carcinoma was made at post-mortem examination.

The third case of alveolar cell carcinoma was diagnosed prior to the lung biopsy as miliary tuberculosis or alveolar cell carcinoma, which was confirmed later by the lung biopsy.

The fourth case was diagnosed as alveolar cell carcinoma on examination of the sputum and the roentgenographic findings, and was later confirmed by lung biopsy.

As alveolar cell carcinoma is not sensitive to X-ray, radiotherapy is not of great help. Surgical treatment in the form of segmental resection, lobectomy, or pneumonectomy in selected cases might prolong or save the patient's life.

Summary

A description is given of 4 patients with alveolar cell carcinoma, one male and 3 females, aged from forty to sixty years. Only one survived.

The clinical, roentgenographic, pathologic, and diagnostic findings are discussed.

Sumario

Carcinoma Alveolocelular

Esta reseña comprende a 4 enfermos, un varón y 3 mujeres, de cuarenta a sesenta años de edad, que tenían carcinoma alveolocelular. Sólo sobrevivió uno.

Se discuten los hallazgos clínicos, radiográficos, histopatológicos y diacríticos.

Resume

Carcinome alvéolaire

Description de 4 cas de carcinome alvéolaire concernant un malade du sexe masculin et trois du sexe féminin dont l'âge variait entre quarante et soixante ans. Un seul a survécu. Les signes

cliniques, radiologiques, pathologiques et diagnostiques sont étudiés.

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Case Reports

PNEUMOLITHS IN PROFUSION IN SILICOSIS[1]

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INTRODUCTION

Indicative of a pathologic end-point of disease, the presence of small, calcific pulmonary densities or pneumoliths in great number is an uncommon manifestation of many diseases. When noted in the chest roentgenogram, usually these opacities are attributed to such infections as miliary tuberculosis and histoplasmosis. Less commonly they are regarded as evidence of such diverse conditions as rheumatic fever, with or without valvular heart disease; hemosiderosis due to mitral stenosis; fungal diseases other than histoplasmosis; and hypercalcemia. Rarely are they ascribed to the larval stage of ascariasis, pulmonary alveolar microlithiasis, and silicosis.

That silicosis should be regarded as a quite unusual etiologic factor of widely disseminated pneumoliths is borne out by the experience of the Wilkes-Barre Veterans Administration Hospital, despite its location in a hard-coal mining region where literally hundreds of miners are admitted annually. Since the opening of the hospital approximately eight years ago, only one case with these roentgenographic findings attributable to silicosis has been encountered. This case forms the basis of the present report.

CASE REPORT

The first admission to the Wilkes-Barre Veterans Administration Hospital of the 68-year-old white male miner was on September 13, 1958. He had worked in the hard-coal mines, where he was exposed to silica and coal dusts, for a period of thirty-six years prior to 1948; since 1948 he had been unemployed. He had the following chief complaints: (*1*) cough and expectoration, (*2*) loss of weight, (*3*) swelling of the feet, and (*4*) dyspnea on slight exertion. For many years he had had cough and expectoration. The cough, more pronounced in the evening, had been productive of a moderate amount of tenacious, yellowish sputum. For the past two years he had been gradually losing weight, the loss of weight being approximately twenty pounds; this symptom was associated with anorexia for the same period of time. During the past year, he had noted swelling of both feet, particularly in the evening but subsiding after a night's rest. The most troublesome symptom was exertional dyspnea, noted initially about two years before admission to the hospital. Since its onset the dyspnea has so progressed that it became manifest several months ago on walking a short distance on level ground.

The patient denied such symptoms as hemoptysis, paroxysmal nocturnal dyspnea, and substernal pain. In addition he had no history suggestive of miliary tuberculosis, rheumatic fever, helminthiasis, excessive intake of vitamin D, or of exposure to any dust other than silica and coal.

Physical examination on admission revealed a 68-year-old white male, fairly well nourished, chronically ill, and slightly dyspneic at rest. His height was 64 inches; weight, 114 pounds; temperature, 98°F.; pulse regular in force and rhythm with a rate of 96 per minute; and blood pressure 125 systolic, 90 diastolic in mm. of mercury. The chest showed an emphysematous configuration, with the respiratory excursions limited on both sides. The lungs were hyperresonant to percussion, the breath sounds were distant, and scattered expiratory rhonchi were audible throughout both lung fields. Cardiac examination showed nothing abnormal. Pedal pulsations were found on both sides and there was no evidence of pedal edema. The remainder of the physical examination revealed no significant abnormalities.

The results of the following determinations were normal: urine, complete blood count, blood urea nitrogen and sugar, serum albumin and globulin, serum bilirubin, blood cholesterol and esters, prothrombin time, thymol turbidity, cephalin flocculation, serum calcium, phosphorus, and alkaline phosphatase. A serologic test for syphilis was negative. Several sputum specimens examined by microscopy and culture revealed no acid-fast bacilli. Sputum specimens also examined by culture for fungi revealed no growth. Two smears of the peripheral blood for lupus erythematosus cells were negative. There were no ova or parasites in the stools. The collodion agglutination and complement-fixation blood tests were negative for histoplasmosis, blastomycosis, and coccidioidomycosis (these tests were performed at the Walter Reed Army Institute of Research, Department of Bacteriology, Washington, D. C.). The electrocardiogram was normal.

A film of the abdomen showed no calcific densities. A gastrointestinal series and barium enema studies, done because of loss of weight and anorexia, revealed no evidence of disease. A chest film on March 28, 1950 (figure 1) which was ob-

[1] From the Medical Service, Veterans Administration Hospital, Wilkes-Barre, Pennsylvania.

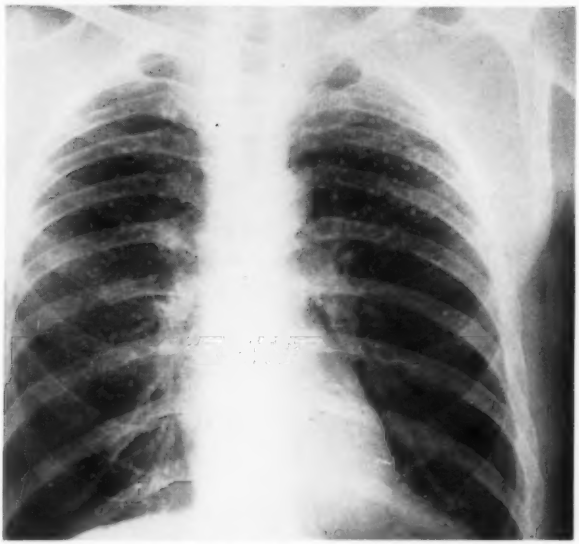

FIG. 1. Roentgenogram (1950) showing bilateral calcific densities and emphysema at the bases.

FIG. 2. Roentgenogram (1958) is similar to figure 1 except for new calcific densities in the right upper lobe.

tained after the patient was hospitalized, demonstrated small calcific densities up to 3 mm. in size throughout both lung fields except at the bases, where there was evidence of emphysema. A chest roentgenogram obtained on September 15, 1958, the hospital film (figure 2), is similar to the previous one except for the presence of new calcific densities in the right upper lobe. A Bucky roentgenogram of the chest October 10, 1958 (figure 3), over-exposed relative to the usual exposure of a chest film, still shows the calcific densities. A tomogram of the chest, 7 cm. from the back, obtained on October 7, 1958 (figure 4), shows not only the calcific densities in the lungs, but also a calcific

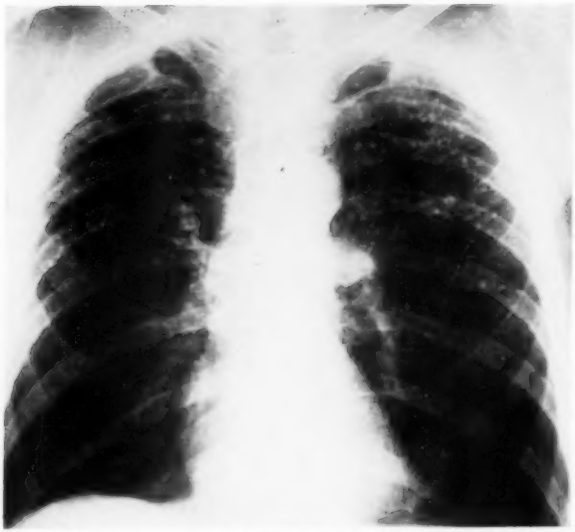

FIG. 3. Bucky roentgenogram (1958) still showing the calcific densities.

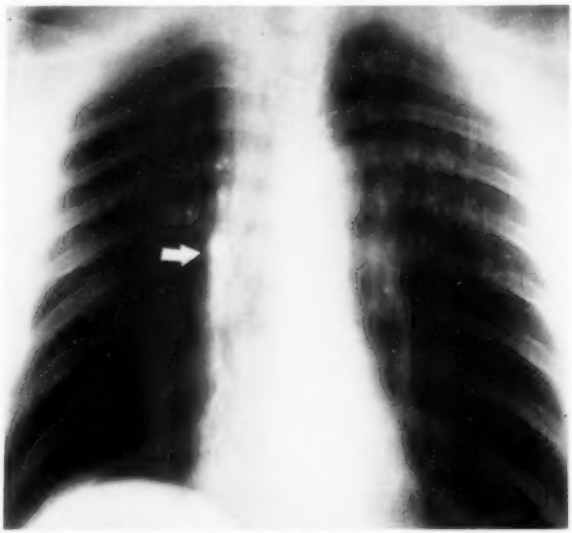

FIG. 4. Tomogram of the chest (1958) showing pulmonary calcific densities and a rounded, relatively large, calcific nodule in a right hilar node.

density in a hilar node on the right; this density is most prominent in the tomogram of the figure.

A skin test with purified protein derivative, first-strength, was positive. Histoplasmin and coccidioidin skin tests were negative. On September 25, 1958, a scalene node biopsy on the right was performed. Only fibro-fatty tissue without any lymphoid tissue was noted by microscopy.

Pulmonary function studies, performed after the patient had improved on therapy, revealed marked impairment in pulmonary ventilation, obstructive in type. This finding is compatible

with chronic bronchitis and generalized obstructive emphysema.

In the several weeks while the patient was hospitalized, he was on a regular diet and treated with bronchodilators in the form of aminophylline, isoproterenol (Isuprel®), and ephedrine. He also received a short course of penicillin. On this therapy the patient improved: his cough and expectoration became minimal and he was no longer dyspneic at rest. He was essentially afebrile and he gained three pounds in weight.

Comment: On the basis of the evidence just recorded, the final diagnoses made were: (*1*) pneumolithiasis attributable to silicosis, and (*2*) chronic bronchitis and pulmonary emphysema. It was believed that bronchial irritation due to the prolonged inhalation of both silica and coal dust (like that of coal dust alone in coal-workers' pneumoconiosis (1)), not the nodules, probably accounted for the chronic bronchitis and pulmonary emphysema. The diagnosis of these conditions was substantiated by clinical and roentgenographic findings and pulmonary function studies. The reasons for the diagnosis of pneumoliths due to silicosis will be given in the discussion. That these densities are calcific is manifested by their marked radiopacity in all roentgenograms.

Although helpful diagnostically if the tissue removed is examined not only by microscopy and culture but also by chemical analysis and roentgenographic diffraction, pulmonary biopsy was not performed for two reasons: the risk attendant on such a procedure in a patient with markedly reduced pulmonary function, and a belief that the result of the examination of tissue removed would probably not lead to any change in therapy since the pneumoliths were regarded as an end-stage of silicosis.

Discussion

As mentioned in the Introduction, many diseases of diverse etiology may lead to the appearance of numerous pneumoliths. Consideration of the circumstances under which calcification occurs indicates that a number of these diseases may have basic pathologic features common to all of them.

As is well known, calcification is a phenomenon occurring in the development and growth of the human body. Normally calcium salts are deposited in the bones and teeth; but in certain tissues, such as the pineal body, the deposition may also be regarded as a normal event.

Just as well known is that calcification—abnormal calcification—occurs in other sites, but as a result of disease. According to Ogilvie (2), lime salts may be deposited in tissues either because of hypercalcemia as the result of hyperparathyroidism or vitamin D overdosage, for example; or because such tissues are the site of specific disease processes. In hypercalcemia, the lungs, kidneys, and stomach are usually the sites of such deposits. The explanation for this occurrence is that there is an increased alkalinity of these tissues resulting from their excretion of acids. With regard to specific disease processes, he states that lesions demonstrating either hyaline change or necrosis may become calcific. Of the various types of pathologic calcification, Anderson (3) is of the opinion that necrotic lesions are the most frequent sites of lime deposition.

Is necrosis then seen in various pulmonary diseases in which calcification may supervene? In ascariasis, death of widely disseminated larval forms of the helminth causative of this disease may occur. In rheumatic fever and hemosiderosis of mitral valvular disease, it is more probable that hyalinization precedes calcification since necrosis of significance is not described as occurring in these conditions. In miliary tuberculosis, histoplasmosis, and other fungal diseases, necrosis is seen; and this process has also been observed by Gardner (4) in experimental silicosis.

Not commented upon as yet is the deposition of lime salts in pulmonary alveolar microlithiasis. Two hypotheses have been advanced for this deposition: increased alkalinity of the lung tissues resulting from the excretion of carbon dioxide, and this in the absence of hypercalcemia; and result of tissue damage due to inhalation and deposition of dust particles. In the review of seventeen cases of this entity, including one reported by them, Finkbiner and his colleagues (5), found in six a history of dust exposure. It may well be that this disease in some cases may be the result of tissue necrosis due to a harmful dust and subsequent calcification.

That calcification may occur in silicotic nodules is attested to by both experimental and clinical evidence to which speculation, interesting if nothing else, is added. According to Gardner (4), whose work has already been briefly mentioned, excessive concentrations of silica particles, particularly in rabbits, may cause necrotic pulmonary lesions with subsequent calcification. In

this regard, the possibility exists that an excessive deposition of hemosiderin, which may be looked upon as causing an endogenous harmful pneumoconiosis in man, can produce necrosis followed by lime deposition. On theoretic grounds, it is conceivable that in man individual variation in the direction of undue sensitivity of the lung to silica may also lead to a similar chain of events. Furthermore, Gardner (4), and Twining and Kerley (6), as well, report the observation of a few human cases of silicotic pneumolithiasis.

In addition, Twining and Kerley state that, if pneumoliths are the result of silicosis, usually round calcified nodules are noted in the hilar nodes of both sides. They are not referring to the egg-shell calcification in silicotic hilar nodes which may be seen without pulmonary calcification. In the case reported, a rounded, calcified nodule was seen only in the right hilum (*see* figure 4).

Three explanations for the infrequency of calcification in human silicosis can be listed: the characteristic lesions of silicosis are hyaline nodules which are not as apt as necrotic lesions to attract lime salts; pulmonary deposition of silica sufficiently high to cause necrosis rarely occurs; and undue sensitivity to silica deposition in the lungs, as manifested by necrosis, is probably a rare phenomenon.

Discussion of the clinical differentiation of the diseases leading to pneumolithiasis entails the consideration of clinical, laboratory, and roentgenographic evidence. In this discussion consideration will also be given to such evidence, or lack of it, in the case of this report which justified the diagnosis which was made. It might be added that this case may be regarded as generally representative of silicotic pneumolithiasis.

One might begin with the roentgenogram since it is absolutely essential to the clinical diagnosis of pneumolithiasis. Initially it must be decided whether these densities are actually the reflection of calcium salts or that of other substances capable of throwing shadows comparable to the radiopacity of calcium salts. It should not be forgotten that in the benign pneumoconioses due to such dusts as the oxides of tin, iron, or barium, whose molecular weights are quite high, opacities just as dense as, or denser than, those produced by calcium salts are visible. In the case reported there was no exposure to any dust other than silica and coal.

Once it is decided that the densities are due to calcium deposits, then the size and location of these deposits in the roentgenogram are of importance in the differential diagnosis. In pulmonary alveolar microlithiasis and lithiasis of hypercalcemia, the calcific opacities are minute and have a stippled appearance. In rheumatic fever (7) or hemosiderosis of mitral stenosis (8), the nodules, although similar in size to those of the reported case, are located usually in the bases of the lungs. In the reported case the nodules were only apparent in the upper two-thirds of the lung fields, scattered calicific lesions in the bases, if present, being obscured by the emphysema present.

Furthermore, the roentgenogram is of great importance from another point of view. If, in the face of an adequate occupational history, the calcified nodules are the only pulmonary lesions in profusion, then these nodules can probably be attributed to silicosis. In the reported case no other pulmonary opacities were encountered.

The clinical and laboratory findings must also be considered in viewing the background of pneumolithiasis. In hypercalcemia, histoplasmosis and other fungal diseases, ascariasis, and rheumatic fever with or without valvular disease—in contrast to silicosis—an adequate occupational history and evidence of chronic bronchitis and emphysema are lacking. In addition, hypercalcemia is manifested by specific chemical changes in the blood and possible evidence of calcification in other organs, findings which were not seen in the reported case.

The clinical diagnosis, therefore, of plentiful pneumoliths attributable to silicosis can be made on the following grounds: an adequate occupational history, exclusion as far as possible of any other disease in which similar lesions may occur, and absence of other pulmonary evidence of silicosis on the chest roentgenogram.

One other finding requires clarification: the appearance of new calcific densities in the chest film of 1958 as compared to that of 1950, since the patient was not exposed to dust after 1948. It has been shown by a special technique that silica may migrate from hyaline nodules to unaffected areas of the lung, where presumably new nodules may be formed (9). Similarly, it is conceivable that silica particles may just as well move out of calcific nodules to lead to the formation of other calcific lesions.

Summary

An unusual case has been reported demonstrating pneumoliths in profusion attributable to silicosis. This case has been termed unusual since such lesions are rarely seen in silicosis.

The diagnosis in this case, made clinically, was based upon the following considerations: an adequate occupational history; exclusion of other disease in which similar densities may occur; and absence of other pulmonary evidence of silicosis on the roentgenogram.

Sumario

Profusión de Neumolitos en la Silicosis

Se describe un caso extraño que revelaba una profusión de neumolitos imputables a la silicosis. Se denomina el caso extraño por observarse rara vez esas lesiones en la silicosis.

El diagnóstico en este caso, formulado clínicamente, se basó en los siguientes puntos: adecuada historia profesional; exclusión de otras dolencias en que pueden presentarse condensaciones semejantes; y falta de otros signos pulmonares de silicosis en la radiografía.

Resume

Profusion de pneumolithes dans la silicose

L'auteur rapporte un cas exceptionnel démontrant la présence d'une profusion de pneumolithes imputables à la silicose. Ce cas a été qualifié d'exceptionnel car ces lésions sont rarement vues dans la silicose. Dans ce cas, le diagnostic clinique était fondé sur les considérations suivantes: des antécédents professionnels adéquats, l'exclusion d'autres maladies dans lesquelles des opacités similaires peuvent apparaître, l'absence d'autres signes de silicose pulmonaire sur les radiogrammes.

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Notes

THE EFFECT OF ISONIAZID UPON THE GROWTH OF TUBERCLE BACILLI FROM PULMONARY LESIONS

The use of surgical resection and chemotherapy in pulmonary tuberculosis has given rise to many new problems. One of the most puzzling and important questions has been the fate of the acid-fast bacilli which continue to be visible in unsloughed nodular lesions. D'Esopo and co-workers,[1] as well as Hall and Gleason,[2] found that, in cases treated daily with streptomycin and PAS, tubercle bacilli were often seen in smears from resected nodules, but these lesions yielded only a small percentage of positive cultures on inspissated egg medium. Similarly, Canetti reported a much lower proportion of culturable bacilli in closed caseous lesions than in open cavities in patients given isoniazid, streptomycin, or PAS.[3] D'Esopo and associates concluded that chemotherapy and closure or inspissation of necrotic lesions brought about death of these bacilli.[4] Similar observations and conclusions were made by several other investigators.[5-8]

In 1954, a new method for culture of tissues which was designed to dilute out materials toxic to multiplying tubercle bacilli was introduced by Hobby and associates.[9] In their hands this method resulted in a high percentage of positive cultures, expecially after prolonged incubation. Tubercle bacilli were isolated from 25 of 31 tuberculous pulmonary lesions. Nine of these lesions showed culturable bacilli only after the culture was held more than sixty-three days. They made no attempt to compare their method with conventional methods of culture. Nine of the patients had received isoniazid, and in eight, culturable tubercle bacilli were found. Each of these eight patients had a cavitary lesion. In a subsequent report, these investigators showed that the recovery of tubercle bacilli from "closed" nodular lesions was closely correlated with their stage of healing.[10]

The purpose of this paper is to report results on the recovery of tubercle bacilli from pulmonary lesions removed after multiple-drug therapy, including isoniazid. The tissues were prepared by the method of Hobby and co-workers.[9] Cultures were made in both Dubos' liquid medium and Löwenstein-Jensen medium. Guinea pigs were also inoculated.

Seventy-three tuberculous lesions from 65 patients with consecutive surgical pulmonary resections following multiple-drug therapy were studied. Of the 65 patients, 54 had been previously untreated. Eleven of the patients represented re-treatment cases. Three nodules from 3 patients who had received no antituberculous chemotherapy were also studied.

The chemotherapeutic regimens in the original treatment cases were: streptomycin, 1 gm. intramuscularly twice weekly, plus PAS, 12 gm. by mouth daily; and isoniazid, 300 mg. orally daily along with either streptomycin twice weekly or PAS daily.

The lesions included in this study were classified as either nodular or cavitary. The classification of nodular lesions, based on the degree of healing, was adopted exactly from Auerbach and associates,[10] except that inspissated cavities were not included. The nodular lesions were subdivided into three groups: *active inflammation*, *intermediate healing*, and *advanced healing*, depending upon the degree of hyalinization of the connective tissue surrounding the lesion and the deposition of calcium within the necrotic zone. The cavitary lesions were classified as "open active," "open healed," fluid filled, and inspissated. A classification of the lesions with respect to pathology, culturability of tubercle bacilli, chemotherapy, and recovery of tubercle bacilli is given in table 1.

The method of preparation of the tuberculous

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TABLE 1

CLASSIFICATION OF LESIONS, CHEMOTHERAPEUTIC REGIMENS, AND RECOVERY OF TUBERCLE BACILLI

Lesions	Isolation of Tubercle Bacilli by Culture or Guinea Pig/Number of Lesions					
	Streptomycin PAS		Isoniazid in Combination		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Nodules						
Active	7/18		2/15		9/33	27
Intermediate	0/4		2/13		2/17	12
Advanced healing	—		0/2		0/2	0
Total	7/22	32*	4/30	13*	11/52	21
Cavities						
"Open active"	0/2		1/2		1/4	25
Fluid filled	4/5		0/3		4/8	50
"Open healed"	1/1		—		1/1	100
Inspissated	0/3		1/5		1/8	13
Total	5/11	45†	2/10	20†	7/21	33
All lesions	12/33	36‡	6/40	15‡	18/73	25

* Difference not statistically significant, $\chi^2 = 2.6$, $P < 0.2$
† Difference not statistically significant, $\chi^2 = 1.5$, $P < 0.3$
‡ Difference is statistically significant, $\chi^2 = 4.4$, $P < 0.05$

tissues in this study was that of Hobby and associates[9] with minor modification. The fresh lung specimen was subdivided aseptically. A small portion was set aside for histologic examination and the remainder was used for bacteriologic studies. The latter portion was immediately frozen at −10° C. and stored for one to seven days. The tissue was thawed for two hours at room temperature, weighed, macerated with sterile scissors, and diluted to 5 per cent. The medium used for all dilutions, washings, and cultures was Dubos liquid medium[11] containing 25 units of penicillin per ml. Tween® 80 was omitted from the medium. The specimen was macerated using the Servall High Speed Omni-Mixer.[12] It was then homogenized with a Teflon tissue grinder. The resulting mixture was spun in an International Size 1, Type SB, horizontal-type centrifuge at 1,000 × gravity for thirty minutes. The supernatant fluid was decanted into 250-ml. screw-capped bottles. The sediment was washed three times with diluent; the supernatant fluid from each washing was pooled with the original supernatant fluid. The pooled supernatant mixture was adjusted to a volume of 50 ml. The washed sediment was brought to 1.0 ml. and 0.1 ml. inoculated into each of three tubes of Löwenstein-Jensen egg medium[13] and two guinea pigs. Each guinea pig received 0.1 ml. subcutaneously in the thigh.

All cultures were incubated for nine months unless positive for tubercle bacilli or a contaminant at an earlier date. After one and one-half, three, and nine months, slants of Dubos oleic-acid-albumin agar[11] were inoculated from all tubes of the Dubos liquid medium and incubated for twenty-one days. All positive cultures were confirmed by microscopy, subculture to Löwenstein-Jensen slants, and guinea pig inoculations.

The guinea pigs were tuberculin tested at four weeks and sacrificed after two months. Tuberculous lesions from the liver, spleen, and regional lymph nodes were subjected to culture.

Smears of the macerated tissues and the washed sediment were stained with the Ziehl-Neelsen technique. Stained sections of the lesions were also examined microscopically.

Hobby and associates[9] indicated that, despite prolonged chemotherapy, tubercle bacilli often survive in "closed" necrotic pulmonary lesions. The present study showed a much lower rate of recovery of tubercle bacilli. As noted in table 1, although the tissues were prepared by the same method, tubercle bacilli were cultured from only 21 per cent of 52 unsloughed nodular lesions, 48 of which had been resected from 40 patients who had received a single course of chemotherapy, usually for eight months or longer. By contrast, Hobby and associates cultured tubercle bacilli from 78 per cent of similar lesions.[9] Factors other than the culture method play a role in the recovery of tubercle bacilli from closed nodular lesions.

[11] DUBOS, R. J., AND MIDDLEBROOK, G.: Am. Rev. Tuberc., 1947, *56*, 334.

[12] Manufactured by Ivan Sorvall Inc., Norwalk, Connecticut.

[13] Tuberculosis Laboratory Methods, Appendix B, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, 1955, p. 520.

TABLE 2

RECOVERY OF TUBERCLE BACILLI FROM TUBERCULOUS PULMONARY LESIONS USING DUBOS LIQUID MEDIUM, LÖWENSTEIN-JENSEN MEDIUM, AND GUINEA PIG INOCULATION

Per Cent Positive for Tubercle Bacilli

Lesions	Culture- and/or Guinea Pig-Positive	Positive Culture	Positive Guinea Pig	Negative Culture; Positive Guinea Pig	Positive Culture; Negative Guinea Pig	Positive on Dubos Medium	Positive on Löwenstein-Jensen Medium	Positive on Dubos Medium, Negative on Löwenstein-Jensen Medium	Negative on Dubos Medium; Positive on Löwenstein-Jensen Medium
Nodules	22 (12/55)*	16 (9/55)	13 (7/55)	5.5 (3/55)	9.1 (5/55)	11 (6/55)	15 (8/55)	1.8 (1/55)	3.6 (2/55)
Cavities	33 (7/21)	29 (6/21)	19 (4/21)	4.8 (1/21)	14 (3/21)	24 (5/21)	14 (3/21)	14 (3/21)	4.8 (1/21)

* Number of lesions positive/number of lesions tetsed.

Prolonged incubation has been stressed as desirable in the culture of pulmonary lesions by the Hobby method. In the original report,[9] 9 of 25 lesions were positive only after more than sixty-two days (sixty-three to two hundred twenty-three days). Delayed growth occurred for the most part with tissues from re-treatment cases. Of the 19 positive lesions in the present study, 16 were positive after less than sixty days. Two of the specimens were positive on Löwenstein-Jensen slants between the sixtieth and seventieth days, and one specimen, positive in Dubos liquid medium, required between ninety and one hundred days of incubation. These results indicate that prolonged incubation may not be necessary with the Hobby technique except in re-treatment cases. Culturable tubercle bacilli present in a tissue specimen may be missed by most present methods, however, if the culture is incubated for less than two months.

Culturable tubercle bacilli were found in 27 per cent of active nodular lesions and only 12 per cent of those showed intermediate healing (*see* table 1). Two nodules with advanced healing proved sterile. Auerbach and co-workers[10] found culturable tubercle bacilli in 100, 43, and 14 per cent, respectively, of similar lesions. The difference in results is not explained by the fact that inspissated cavities were excluded from this group of nodular lesions, for only one of 8 such lesions was positive in this study. However, 4 of 8 fluid-filled cavities contained culturable tubercle bacilli. Lesions that have sloughed and filled may contain more culturable bacilli than unsloughed nodules, but they tend to become sterile when inspissated. Attainment of D'Esopo's "target point"[1,14] did not assure healing, for 23 of 37 nodules in such cases were active as judged microscopically. Furthermore, 16 per cent showed culturable tubercle bacilli. In patients on isoniazid regimens and at the target point, nodular lesions showed microscopic evidence of healing more often than in patients who received no isoniazid (*see* table 1).

The effect of the duration of the preoperative chemotherapy on the recovery of tubercle bacilli was studied in 52 nodular lesions, 37 of which were from target-point cases. Of 7 target-point patients on 1 gm. of streptomycin twice weekly and 12 gm. of PAS daily for eight months or more, 43 per cent contained culturable tubercle bacilli. In 7 target-ipont patients who had been on a regimen containing 300 mg. of isoniazid daily for eight months or more no culturable tubercle bacilli were found.[15] There was not a significant difference in the recovery of tubercle bacilli from nodules in target-point patients treated either zero to seven, or eight or more, months with antituberculous drugs. Even in patients who had received isoniazid, stainable bacilli were common in unsloughed nodular lesions (54 to 100 per cent).

In table 2 the results are recorded of a comparative study of three methods for culturing tubercle bacilli from pulmonary lesions prepared by the Hobby technique. The methods were: Löwenstein-Jensen slants, Dubos liquid medium, and guinea pig inoculation. The results indicate that cultural methods generally were more sensitive in recovering tubercle bacilli than was guinea pig inoculation. However, guinea pig inoculation appeared more sensitive with lesions containing few or no stainable bacilli. No significant difference was noted in the recovery of tubercle bacilli from nodules when the two culture media were compared.

Acid-fast bacilli were detected by microscopic techniques in 64 of the entire group of 76 lesions.

[14] Sputum cultures negative for tubercle bacilli, cavities closed, and maximal roentgenographic clearing.

[15] The difference is statistically significant, $P < 0.01$.

Acid-fast bacilli were found more often in microscopic sections than in smears from the homogenized tissues. However, the percentage of acid-fast positive lesions from which tubercle bacilli were cultured by either stain technique was the same, only 27 per cent.

* * *

A comparative study of the preparation and culture of tissues by the Hobby method in Dubos liquid medium and the Veterans Administration method on American Trudeau Society inspissated egg medium was undertaken by Raleigh and associates.[16] Their study included 68 patients with 168 resected pulmonary lesions. Tubercle bacilli were cultured in only 16 per cent of 25 target-point nodules by the Veterans Administration method, and in 12 per cent by the Hobby method.

The present studies with the Hobby method did not approach the success which was attained in the original study.[9] This does not imply that the method is not valuable for the recovery of latent bacilli from tissues. The differences in the results probably depend on several factors. The stage of closure, inspissation, and healing of the lesions resected may have been different and the chemotherapeutic regimens employed were not identical. Such discrepancies in results as have been observed in different laboratories could perhaps be resolved by an exchange of "unknown" specimens and, as far as the writers know, no such comparisons have been published.

The influence of the type of chemotherapy on the culturability of tubercle bacilli from lesions containing acid-fast bacilli on microscopy continues to present an unsolved problem. Beck and Yegian[7] point out that, even when no chemotherapy has been used, difficulty may be encountered in culturing tubercle bacilli from some caseous lesions. Canetti stated that chemotherapy does not appreciably affect the culturability of tubercle bacilli contained in unsloughed necrotic lesions.[3] The present findings, however, suggest that a regimen containing isoniazid may reduce the chance of culturing tubercle bacilli from these lesions. It was impossible to ascertain whether the reduction in the number of positive cultures was due to the recent chemotherapy per se or to the fact that the lesions had healed more rapidly as a result of the therapy. However, the more frequent finding of culturable tubercle bacilli in unsloughed nodules in cases at target point after streptomycin and PAS supports the idea that isoniazid may "sterilize" such lesions and lead to earlier healing although some visible tubercle bacilli remain. The metabolism and virulence of such organisms may have been so altered as to render them dormant and not necessarily all dead.

In summary: Tuberculous lung lesions, mostly nodular, were resected after treatment with streptomycin and PAS or isoniazid with PAS or streptomycin. Although 36 per cent of the lesions contained culturable tubercle bacilli in the streptomycin-PAS cases, only 15 per cent of the lesions from patients who had received isoniazid had positive cultures. The unsloughed nodules in isoniazid cases showed more advanced healing. No significant difference was found in the incidence of culturable tubercle bacilli in tuberculous lesions by inoculation into guinea pigs, Löwenstein-Jensen medium, or Dubos liquid medium (without Tween 80). Prolonged incubation of the cultures (nine months) did not greatly increase the number of positive cultures in the previously untreated cases.

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[16] RALEIGH, J. W., *et al.*: Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, 1955, p. 187.

SERUM PROTEIN ELECTROPHORETIC PATTERN AND MIDDLEBROOK-DUBOS TITER IN TUBERCULOUS CHILDREN FOLLOWING BCG VACCINATION

Although a large body of information has been accumulated in recent years concerning the biologic aspects of tuberculosis, understanding of some of the most fundamental problems relating to hypersensitivity and to immunity in this disease is far from clear.

In a previous report, the present writers have shown that, when children vaccinated with BCG vaccine are given isoniazid or isoniazid and cortisone simultaneously, they fail to develop tuberculin hypersensitivity, detectable with at least 10 TU of purified protein derivatives (PPD),[1] while they do regularly produce antibodies against the polysaccharides of *M. tuberculosis* (Middlebrook-Dubos titer). It is true that local reaction to tuberculoprotein is closely related to the degree of allergy. However, while it has been shown that the infected body produces antibodies against the various chemical constituents of *Mycobacterium tuberculosis*, at the present time no antibody titer can be used with certainty as a diagnostic tool or as an indication of the degree of acquired resistance against tubercle bacilli. On the contrary, it appears that the changes in the electrophoretic patterns of serum protein in tuberculosis are rather characteristic and may be correlated with the evolution of the disease.[2]

The present investigation was undertaken with the purpose of studying the electrophoretic changes in serum protein and the Middlebrook-Dubos titer, as well as any correlation between them, in tuberculous children undergoing an intense antigenic stimulus with BCG vaccine.

Twenty-five infants and children aged three months to twelve years, suffering from various forms of febrile primary tuberculosis (9 patients with enlarged lymph nodes only and 16 with enlarged nodes plus consolidation/collapse of one or more segments) were vaccinated intradermally in the deltoid region of the left arm with 0.1 ml. of BCG vaccine prepared by the Institut Pasteur Hellenique. Before vaccination and twenty-four, forty-eight, and seventy-two hours, and seven days following it, all children were examined roentgenographically and blood was drawn for electrophoretic studies of serum protein fractions and Middlebrook-Dubos titer determinations. No patient was given any kind of chemotherapy. Five nontuberculous children, negative to 10 TU of PPD, were also vaccinated with BCG and observed as controls.

In all controls no change whatsoever was noted either locally or in the electrophoretic pattern of serum protein determinations and Middlebrook-Dubos titer during the seven days of observation following vaccination with BCG (table 1). Within forty-eight hours following the vaccination with BCG vaccine, all of the 25 tuberculous children developed typical Koch phenomenon at the site of vaccination, consisting of erythema and induration of a mean diameter of 22 mm. and 19 mm., respectively. Six children also developed transient hyperpyrexia twenty-four hours after the vaccination. Two more children with extremely enlarged nodes and consolidation/collapse of one bronchopulmonary segment exhibited definite roentgenographic aggravations seven days following the vaccination. The changes in the electrophoretic pattern of serum protein fractions and fluctuations of Middlebrook-Dubos titer are shown in table 2. The albumin/α_2 ratio is shown in figure 1.

Statistical analysis of the differences in the values of albumin and the globulin fractions obtained before and after vaccination showed that they were significant only as far as the albumin and the α_2-globulin fraction were concerned. For differences in the values of albumin, $t \geq -4.97$, -4.97, and -3.53 at twenty-four, forty-eight, and seventy-two hours, respectively [Pr. ($t \leq$ values obtained) < 0.5 per cent].

For differences in the α_2-globulin fraction, $t =$ 5.10, 8.3, and 3.7 at twenty-four, forty-eight and seventy-two hours, respectively [Pr. ($t \geq$ values obtained) < 0.5 per cent].

On the contrary, t at seven days after vaccination was -1.03 for albumin [Pr. ($t \leq -1.03$) > 15 per cent] and 0.93 for α_2-globulin [Pr. ($t \geq 0.93$)$>$ 15 per cent] and is consequently statistically insignificant.

When the same statistical method was applied to the other fractions, the differences from prevaccination values were shown to be insignificant.

* * *

As expected, an accelerated type of local inflammation (Koch phenomenon) was produced in all patients at the site of vaccination. The roentgenographic aggravation which was observed in 2 children seven days following the vaccination may be attributed to an increased local exudative reaction which occurred under the influence of a

[1] Choremis, C., *et al.*: Am. Rev. Tubere., 1956, 74 (Supplement, p. 197).

[2] Gilliland, I. C., *et al.*: Brit. M. J., 1956, No. 4981, 1460.

strong antigenic stimulus by the tuberculoprotein of the BCG vaccine.[3]

As was shown twenty-four hours following the vaccination, a significant fall of serum albumin and rise of α_2 globulin fraction occurred in all patients and persisted for forty-eight hours more. Subsequently, a gradual increase of albumin and fall of α_2 globulin commenced. By the seventh day after the vaccination the electrophoretic pattern of serum protein resembled closely the one present before vaccination. The albumin/α_2 ratio, which seemed to be a more sensitive index than the individual changes of these two fractions, fell significantly twenty-four hours after vaccination and returned to prevaccination levels only after seven days.

Similar changes in the electrophoretic pattern of serum protein, i.e. decrease in albumin and increase in α_2 and γ-globulin fractions, have been consistently observed in tuberculous patients and were closely associated with the evolution of the disease, since a gradual approach toward normal figures accompanied clinical improvement. All of the 25 tuberculous children included in this study exhibited changes in the electrophoretic pattern of their serum protein before vaccination with BCG. As has been shown, these changes, with the exception of the γ-globulin fraction, were accentuated shortly after vaccination but gradually disappeared. By the seventh day following vaccination, the serum protein electrophoretic pattern became almost identical with the one present before vaccination.

The nature of the findings described is rather obscure. That they do not represent a simple hypersensitivity reaction, that is, an allergic response to tuberculoprotein, was shown by the ineffectiveness of Old Tuberculin[4] in producing any change in the electrophoretic pattern of serum protein in tuberculous children. Thus, it appears that the entire bacillary body is required for the creation of this phenomenon. The Middlebrook-Dubos hemagglutination reaction which was positive to a mean dilution of 1:9 prior to the vaccination

TABLE 1

MEAN VALUES OF SERUM PROTEIN FRACTIONS AND MIDDLEBROOK-DUBOS TITER IN FIVE NONTUBERCULOUS CHILDREN BEFORE AND AFTER BCG VACCINATION

	Serum Proteins					Middlebrook-Dubos Titer
	Albumin	Globulins				
		α_1	α_2	β	γ	
Before vaccination	3.90	0.28	0.64	0.75	1.45	(−)
After vaccination:						
24 hours	3.94	0.29	0.59	0.81	1.42	(−)
48 hours	3.93	0.29	0.62	0.78	1.44	(−)
72 hours	3.92	0.29	0.63	0.75	1.39	(−)
7 days	3.96	0.27	0.60	0.73	1.42	(−)

TABLE 2

MEAN VALUES OF SERUM PROTEIN FRACTIONS AND MIDDLEBROOK-DUBOS TITER IN 25 TUBERCULOUS CHILDREN BEFORE AND AFTER BCG VACCINATION

	Serum Proteins					Middlebrook-Dubos Titer
	Albumin	Globulins				
		α_1	α_2	β	γ	
Before vaccination	2.89	0.37	0.74	0.92	1.53	1:9
After vaccination:						
24 hours	2.67	0.35	0.93	0.87	1.45	1:20
48 hours	2.86	0.35	0.92	0.87	1.45	1:25
72 hours	2.86	0.35	0.84	0.86	1.45	1:32
7 days	2.99	0.37	0.78	0.93	1.54	1:26

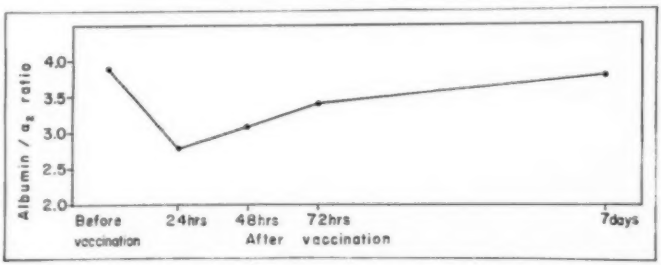

FIG. 1. Albumin/α_2 ratio in 25 tuberculous children before and after vaccination.

[3] CHOREMIS, C. B., *et al.*: Am. Rev. Tuberc., 1956, *72*, 527.

[4] CHOREMIS, C., AND NICOLAIDOU, M.: Unpublished data.

rose to 1:32 seventy-two hours following vaccination and subsequently showed a gradual decline. The rise in antibody titer following vaccination with BCG vaccine appears to be specific and associated with simultaneous changes in the electrophoretic pattern of serum protein. Cole and Favour[5] have shown that the entire Middlebrook-Dubos titer, namely, the antibodies against the tuberculopolysaccharides, belongs to the γ-globulin fractions; and this was unquestionably proved by the inability of persons with congenital agamma-globulinemia to produce such antibodies although they can develop tuberculin hypersensitivity.[6] It seems that these antibodies occupy only a minute portion of the γ-globulin fraction, however, since the aforementioned antibody titer rise, although significant by itself, was not accompanied by a parallel increase of the γ-globulin fraction in the present cases.

The findings reported here, namely transient decrease in albumin and increase in α_2-globulin fraction as well as increase in the Middlebrook-Dubos titer exhibited by tuberculous children following vaccination with BCG vaccine, resemble very much the ones expected in a heavy tuberculous infection. However, these transient biologic changes do not seem to alter the enduring allergic state of the host. It remains to be seen whether its acquired resistance is in any way influenced by the serologic changes.

In summary: Twenty-five tuberculous children were vaccinated with BCG, and the electrophoretic pattern of serum protein and Middlebrook-Dubos titer were determined before and after the vaccination. A significant but transient fall in albumin and rise in α_2 globulin as well as a gradual but definite increase in Middlebrook-Dubos titer were observed.

Addendum

Since submitting this manuscript, a second BCG vaccination was done eight days after the initial one, when the electrophoretic pattern of serum proteins had already returned to pre-vaccination levels, and the experiment was repeated exactly as described in this paper. This second antigenic stimulation was applied to 10 tuberculous children who- curiously enough—did not respond in the same way as following the first BCG vaccination. Their serum protein electrophoretic pattern remained quite unchanged while the Middlebrook-Dubos antibody titer increased still further.

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[5] Cole, L., and Favour, C.: J. Exper. Med., 1955, *101*, 391.

[6] Herbert, M., and Porter, M. D.: Pediatrics, 1957, *20*, 958.

[7] The writers are grateful to K. Kallergis for undertaking the statistical analysis.

BRONCHOGRAMS UNDER HYPNOSIS

At Muirdale Sanatorium it became necessary recently to do many more bronchograms than in previous years. This was brought about by the fact that the surgeon was seeking associated bronchiectasis with tuberculosis.

The method formerly employed in bronchography was to premedicate the patient and use tetracaine (Pontocaine®) to anesthetize the throat; then, a catheter was passed either with ease or great difficulty into the trachea and more anesthetic had to be used. The principal difficulties with this procedure were an occasional tetracaine death, and coughing throughout the procedure.

About twelve months ago, it was decided to study the feasibility of obtaining bronchograms under hypnosis. By this method, anesthesia would no longer be necessary, and there would be no coughing during the bronchogram. Moreover, it was thought that the time required to execute the procedure might be much shorter.

All of the patients were premedicated with sodium phenobarbital (Luminal®) and tripelennamine (Pyribenzamine®) one to one and one-half hours prior to the start of the procedure as a precaution in the event that the patient was found to be resistant to hypnosis and an anesthetic would have to be employed. There were eleven successful bronchograms obtained under hypnosis in thirteen attempts.

The first patient was studied under post-hypnotic suggestion. She was hypnotized and was told that a bronchogram would be done when she awoke and she would not feel anything in her throat and would not cough. The catheter was passed with ease and the bronchogram obtained with no difficulty or coughing. It was thought that passing the catheter in the post-hypnotic state might be dangerous because the patient's throat might feel anesthetized for a long time and food might be aspirated. It was therefore thought best to obtain the bronchograms under hypnosis.

There was no elaborate preparation or practice prior to the actual procedure. The patients were completely voluntary. The patient was told he was going to be hypnotized and the bronchogram would be obtained, and that there would be no after-effects and nothing would be felt. The ease of induction of the hypnotic state varied from patient to patient. After hypnosis was achieved, the catheter was passed through the nostril into the throat and blindly into the larynx and trachea. The only difficulty encountered was that certain patients had a tendency to cough at the time of the passage of the catheter through the larynx; however, this could be controlled by a single command. It is important to tell the patient that his windpipe is covered with anesthesia, that he feels nothing, that there is no tickling or any feeling in his throat or chest, and that he is completely anesthetized. Propyliodine (Dionosil®) was used as the opaque medium. At first, each side was treated separately; however, subsequently it was found possible to leave the catheter in the trachea and inject both sides at once. The entire procedure was done within fifteen to twenty minutes. The most difficult patients required about thirty minutes for the procedure. Most patients did not recall any part of the whole procedure, although 2 patients stated that they did.

* * *

The bronchograms which were obtained showed detail very well and were accomplished with a minimum of effort. It is realized that in some institutions bronchograms are done without any anesthesia whatsoever. The amount of training in hypnosis that is needed to perform this procedure is not considerable; once under hypnosis, the patient will cooperate.

In summary: Bronchograms can be obtained under hypnosis with relative ease, with no discomfort to the patient, and without fear of toxicity to local anesthetics. There was no risk of reaction to local medication, and very seldom was parenchymal filling noted. When it was found that it was possible to inject both sides at once, the amount of X-ray exposure of the patient was correspondingly reduced.

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QUANTITATIVE ASPECTS OF NEUTRAL RED REACTIONS OF TYPICAL AND "ATYPICAL" MYCOBACTERIA[1]

The tinctorial behavior of mycobacteria treated with neutral red dye and alkaline buffer has been the basis for at least two tests for characterizing members of this genus.[2, 3] In the cytochemical test of Dubos and Middlebrook,[2] a suspension of methanol-washed bacteria is treated with buffered aqueous neutral red solution at a pH of 9.5. Virulent *Mycobacterium tuberculosis* suspensions settle out as a pink sediment, and frank saprophytes settle as a yellow sediment. The so-called atypical mycobacteria give no such clear-cut response to this test. The photochromogens are generally positive, but the nonphotochromogens, or "Battey" types, and the skotochromogens are quite variable and poorly defined by this technique.[4] The microcolonial test, although evolved from the cytochemical test, differs from it in several respects.[2, 5] The microcolonial test cultures are grown on Millipore Filter[6] membrane strips; the colonies are stained, *in situ*, with an acidified alcoholic neutral red solution; they are finally developed, *after* staining, with a five-minute exposure to alkaline buffer. The results of examination of virulent tubercle bacilli or frank saprophytes by this technique agree with the results of the cytochemical test. The atypical mycobacteria, however, uniformly exhibit a positive neutral red reaction in the microcolonial test.[4]

The studies presented here were undertaken to explain the discrepancies in results obtained with the two tests, and with the hope of providing more information for the classification of the atypical mycobacteria.

Bacterial strains employed: Some strains of atypical mycobacteria were selected from the packages distributed by Dr. Ernest Runyon to the participants in the Veterans Administration–National Tuberculosis Association Cooperative Study of Mycobacteria.

Other strains studied were selected from the stock culture collection of the Microbiological Research Unit of this hospital. This collection includes virulent, attenuated, and avirulent strains of mammalian and avian tubercle bacilli, representative strains of the atypical mycobacteria, and stock strains of rapidly growing mycobacteria (including *M. fortuitum*), and of nocardia.

Absorption and retention of dye in the Dubos-Middlebrook cytochemical test: Cultures were grown in duplicate in Tween®-albumin liquid medium. The cultures of rapidly growing mycobacteria, which are neutral-red negative by cytochemical and microcolonial tests, were tested after seven days' incubation at 37°C. These included 2 strains each of *M. fortuitum*, *M. phlei*, and *M. smegmatis*. The cultures of slowly growing mycobacteria, which are neutral-red positive by microcolonial test, and positive or negative by cytochemical test, were tested at fourteen days. Included here were 2 strains of photochromogens, 2 strains of nonphotochromogenic atypical mycobacteria (Battey types), and one culture each of the H37Rv and H37Ra strains of *M. tuberculosis* var. *hominis*.

The cells were concentrated by centrifugation, resuspended in 50 per cent methanol, and incubated at 37°C. for one hour. This was repeated, after centrifugation, with a second 50 per cent methanol wash. One culture of each pair was then suspended in a mixture of 5 ml. of barbital buffer[7] (pH 9.5) and 1 ml. of aqueous neutral red at a concentration of 10 mg. per 100 ml.[8] to yield a final dye concentration of 1.67 mg. per 100 ml. The other culture of each pair was suspended in aqueous neutral red diluted to the same final dye concentration but without barbital buffer. After one hour of incubation at 37° C., all tubes were centrifuged and the color of the sediments recorded.

The suspensions were then washed twice in distilled water to remove free dye and, in the case of the buffered samples, to dilute out the buffer to a neutral pH. All sediments exhibited a pink hue at this point; the intensity of the color was estimated on a scale of 1 to 7-plus. Each plus value on the scale represented a twofold difference in dye bound in the sediment. The scale was based on comparison of sediments with a series of 1 cm.-Schleicher and Schuell 740-E filter paper discs soaked in a series of twofold dilutions of aqueous neutral red, starting with a solution

[1] Presented in part before the Medical Session, as part of *Section 4B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 20, 1958.

[2] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tubere., 1948, *58*, 698.

[3] Wayne, L. G.: J. Bact., 1955, *69*, 92.

[4] Wayne, L. G., *et al.*: Am. Rev. Tubere., 1957, *76*, 451.

[5] Krasnow, I., *et al.*: Am. Rev. Tubere., 1955, *71*, 361.

[6] Millipore Filter Corporation, Watertown 72, Massachusetts.

[7] The barbital buffer consisted of 1 per cent sodium barbital in 5 per cent sodium chloride solution.

[8] Richmond, L., and Cummings, M. M.: Am. Rev. Tubere., 1950, *62*, 632.

containing 1.6 mg. per ml. of the dye (7-plus). (These color standards were useful for about three hours after preparation, after which they dried and faded.)

After the color intensities of the sediments were recorded, all suspensions were mixed with 5 ml. of barbital buffer and incubated again for one hour. These alkaline sediments were then washed twice in distilled water and the intensity of color of sediment again recorded on the 1 to 7-plus scale.

Pre-stain with NR-6: In this technique the cultures were grown as for the cytochemical test. The bacilli were concentrated by centrifugation and resuspended in 0.4 ml. of NR-6 stain. This is an acidified saturated solution of neutral red in ethanol, and stains the sediment very intensely.[4] After ten minutes, the suspension of bacilli in the stain was diluted to 5 ml. with distilled water, and centrifuged. The supernatant fluid was discarded and the stained cells were washed twice more with 5 ml.-portions of water. The washed sediment was resuspended in buffer of the desired pH and observed for color change, i.e., fading of sediment from pink to amber or yellow, at regular intervals ranging from seconds to hours, depending on the pH of the suspending buffer and on the strain of Mycobacterium under investigation.

Roles of absorption and retention of dye in the Dubos-Middlebrook cytochemical test: The results of this study are recorded in table 1. All 6 of the rapidly growing strains gave a negative reaction when treated with the original barbital-neutral red mixture, i.e., the sediments were yellow. The four atypical strains were very weakly positive, i.e., the sediments were amber with a faint tinge of pink. Both strains of *M. tuberculosis* var. *hominis* gave positive reactions. All 12 cultures which were originally treated with the neutral aqueous dye solution stained bright pink, i.e., were 6- or 7-plus.

After treatment of all bacillary sediments with two water washes, no reduction of intensity of hue was observed among the samples originally stained at neutral pH. All sediments originally stained with the alkaline dye solution became pink after washing, but were less intense in color than those stained without buffer. With the exception of the H37Rv strain, which showed a difference of only 1-plus, the alkaline preparations showed a 3 to 6-plus difference in intensity as compared to the neutral preparations. The rapidly growing strains were no different from the atypical mycobacteria in this respect; all exhibited considerably less dye *uptake* from an alkaline solution than from a neutral solution.

The sediments which had been stained originally with unbuffered dye, subsequently incubated with barbital buffer and re-washed with water, were found to have retained as much or somewhat more dye than did those stained originally in the presence of alkali.

Thus alkaline buffer is capable of either: interfering with neutral red absorption by all of the mycobacteria tested, or eluting some dye previously bound (the alkaline buffer solution took on a definite yellow hue after exposure to stained cells). In every case, however, enough dye was bound and retained by the cells to impart a dis-

TABLE 1

EFFECT OF SEQUENCE OF APPLICATION OF REAGENT ON CYTOCHEMICAL REACTION

	Strain Number	Color Index					
		Original Exposure: Alkaline Dye Solution (in Barbital)			Original Exposure: Neutral Dye Solution (in Water)		
		Original Stained Sediment	Washed Sediment	Alkali Treated and Re-washed	Original Stained Sediment	Washed Sediment	Alkali Treated and Re-washed
M. tuberculosis, H37Rv	W-196	Pink	6+*	4+	Pink	7+	5+
M. tuberculosis, H37Ra	P-10	Pink	3+	2+	Pink	6+	5+
M. spp. photochromogen	P-1	Amber-pink	1+	1+	Pink	7+	2+
M. spp. photochromogen	P-16	Amber-white	2+	1+	Pink	7+	4+
M. spp. "Battey" Type	P-2	Amber-pink	4+	1+	Pink	7+	5+
M. spp. "Battey" Type	P-20	Amber-pink	3+	1+	Pink	7+	5+
M. fortuitum	W-201	Yellow	3+	1+	Pink	6+	4+
M. fortuitum	W-202	Yellow	2+	1+	Pink	6+	4+
M. smegmatis	W-254	Yellow	3+	1+	Pink	6+	3+
M. smegmatis	W-289	Yellow	3+	1+	Pink	7+	3+
M. phlei	W-290	Yellow	3+	1+	Pink	6+	3+
M. phlei	P-11	Yellow	4+	1+	Pink	7+	5+

* Each plus-value difference corresponds to a twofold concentration difference of neutral red.

tinct pink hue to the bacillary sediment after the buffer was washed out of the system.

Pre-stain with NR-6: It had been previously observed that atypical mycobacteria, which gave a positive microcolonial test, did fade after prolonged exposure of the membrane strip cultures to alkali. This was not an elution effect, since back flow of reagent from the membrane surface into the reagent pad is negligible. The NR-6 pre-stain test tube technique was developed to study this effect. The very intensely staining NR-6 solution was more satisfactory for these studies than an aqueous dye preparation. The test cultures were stained with NR-6 and water-washed as described above. The buffers to be studied were added to the washed sediments.

The results of one such experiment, designed to relate pH of M/15 phosphate buffer to the fading time of the sediments, are shown in figure 1. The rates of fading of the atypical mycobacteria and of the saprophytes were a function of the pH of the buffer; the atypical mycobacteria, however, were far more resistant to the fading action of alkali than were the saprophytes or *M. fortuitum*, the latter groups fading in seconds and the former requiring minutes to hours. Similar results were obtained with carbonate buffers.

One-tenth molar sodium carbonate solution at a pH of 10.5 was selected for a survey study of a large number of acid-fast organisms by this technique. All strains which faded in an hour or less in the carbonate solution were re-tested, substituting barbital buffer at pH 9.5 for the carbonate.

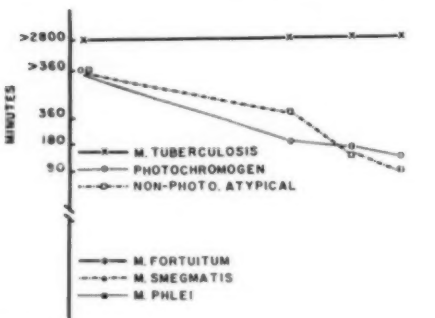

FIG. 1. Time required for fading of pre-stained bacillary sediment after addition of phosphate buffers of various alkaline pH's. Note that the lower half of the logarithmic scale is expressed in seconds, and the upper half in minutes.

The results of this survey are indicated in table 2. *M. tuberculosis* strains resisted the carbonate for more than twenty-four hours. Included here were 2 stock cultures of H37Rv, 2 of virulent bovine bacilli, 3 of H37Ra, 2 of BCG, one of R1Rv, and 2 wild, isoniazid-resistant, catalase-negative, clinical isolates. The photochromogens exhibited a variation in fading time, ranging from two to twenty-four hours; the stock saprophytes, *M. fortuitum*, and the nocardiae all faded quite rapidly, even in the barbital buffer.

The organisms tabulated as wild chromogenic saprophytes have been discussed previously.[4] These are the chromogenic mycobacteria listed by Wayne and co-workers[4] as exhibiting a negative microcolonial test, and which are also negative by guinea pig inoculation (intradermal and subcutaneous routes), and by mouse inoculation (intravenous route). These are probably of no clinical significance.

Three classes of mycobacteria that remain most challenging, from the classification and differentiation standpoint, are *Mycobacterium avium*, the nonphotochromogens (Battey types), and the skotochromogens. Inasmuch as a division had been noted among Battey types on the basis of intradermal pathogenicity for guinea pigs, this information was incorporated into table 2. Battey strains which cause a local ulcerating lesion within three weeks after intradermal inoculation of $\frac{1}{100}$th of a milligram of bacilli[4] (listed in table 2 as *GPid positive*) are, for the most part, only moderately resistant to fading action of alkali and are quite similar in behavior to *M. avium*. In fact, it is not possible to distinguish so-called avirulent *M. avium* and *"Nocardia" intracellularis* from Battey types. Battey strains which do not cause ulceration in guinea pigs in the stated dose (*GPid negative, see* table 2) are, in general, surprisingly resistant to the alkali; they are, in fact, comparable to tubercle bacilli in this respect. The *GPid negative* Battey strains are further distinguished from the *GPid positive* strains in that the former give flaky growth in the Tween-albumin medium (as do the photochromogens), whereas the latter, as well as the *M. avium* strains, grow in a diffuse manner. Among the skotochromogens, no correlation was noted between intradermal pathogenicity and resistance to fading.

* * *

Summarizing relative absorption of dilute neutral red from alkaline solution (*see* table 1) indicates that several factors determine the neutral red reaction of mycobacteria. The ability to ab-

TABLE 2

TIME REQUIRED FOR FADING OF NEUTRAL-RED STAINED MYCOBACTERIA ON EXPOSURE TO ALKALINE BUFFER

	Total Number of Strains Examined	Fading Time					
		Minutes in Barbital (pH 9.5)			Hours in Carbonate (pH 10.5)		
		0–5	5–60	>60	2–6	24	>24
Microcolonial Test Positive:							
M. tuberculosis	12						12
Photochromogens	26				12	14	
M. avium	8		2	5	1		
"Battey" (GPid positive)	27		1	8	15	3	
"Battey" (GPid negative)	12		1			1	10
Scotochromogens	32			10	17	5	
Microcolonial Test Negative:							
M. fortuitum	17	17					
Saprophytes (stock)	14	14					
Chromogenic saprophytes (wild)	23	16	4	3			
Nocardia spp.	9	8	1				

sorb dye from alkaline solution is most pronounced for *M. tuberculosis*, but atypical mycobacteria and saprophytes are also capable of absorbing significant amounts of neutral red from barbital buffer. Some elution of absorbed dye after subsequent treatment with barbital buffer is observed with all types, including *M. tuberculosis*.

When mycobacterial sediments are stained very intensely with concentrated alcoholic neutral red at acid pH, it has been possible to study the effect of alkali on *color* of dye which remains bound. Under these circumstances, the time required to cause the bound dye to fade from red to amber or yellow is a function of pH of the suspending fluid.

The actual time required for fading at a given pH differs widely for different types and species of mycobacteria. Although overlapping of quantitative results of the NR-6 prestaining test precludes precise definition of any single isolate of an atypical Mycobacterium by this technique, consideration of a large number of strains establishes group characteristics. Most saprophytic mycobacteria, *M. fortuitum*, and the nocardiae fade in a matter of seconds or a very few minutes even at a pH as low as 9.5. *M. tuberculosis* requires more than twenty-four hours to fade at pH 10.5. *M. avium* and most of the atypical mycobacteria occupy intermediate positions, closer to the mammalian tubercle bacilli than to the saprophytes.

The Battey types which cause ulcerating lesions on intradermal guinea pig inoculation are culturally indistinguishable from *M. avium*, which also produces ulcerating dermal lesions. Both groups exhibit similar ranges of fading time in the NR-6 prestaining test, although the *M. avium* generally fades a little sooner. Both produce diffuse growth in Tween-albumin broth. Growth rates are comparable for both. Some of the strains received as *M. avium* are not pathogenic for chicks, and these in particular would be difficult, if not impossible, to distinguish from Battey types.

The Battey types which do not cause dermal lesions in guinea pigs in the doses employed have other distinctive properties as well. They are highly resistant to fading in the NR-6 prestaining test. None have been isolated under conditions very suggestive of clinical significance in man, i.e., they were obtained in isolated instances from gastric lavage specimens, but not repeatedly and never from resected tissue. They exhibit granular growth in Tween-albumin broth. They are similar to the "radish" saprophyte reported by Richmond and Cummings[5] and also described by Wayne and co-workers.[4] The single guinea pig-negative strain which faded rapidly in the NR-6 prestaining study (*see* table 2) was isolated in mixed culture from a gastric lavage specimen, along with a skotochromogen. It is possible that this organism represents a nonpigmented mutant of a nonpathogenic skotochromogen. This possibility is under investigation.

It remains to be seen whether the different reactions of various mycobacteria to neutral red are due to quantitative differences in content of a common dye-binding component(s) or to qualitatively different components.

In summary: Quantitative aspects of neutral red reactions of mycobacteria have been investi-

gated by means of modifications of the cytochemical test and the microcolonial test. Even saprophytic mycobacteria absorb significant amounts of neutral red from barbital buffered solutions. On the other hand, some neutral red is eluted from virulent tubercle bacilli by alkali. The alkali interferes with absorption of dye, elutes dye previously bound, and affects the color state of the dye that remains bound.

Intense prestaining of mycobacteria with acidified alcoholic neutral red and subsequent treatment with alkali permits semiquantitative determination of positiveness of cultures, based on pH and time required for fading of bound dye from red to yellow. Group characteristics are observed, *M. tuberculosis* resisting fading the longest, followed, in order, by: photochromogens; *M. avium*, "Battey" types, and skotochromogens; saprophytes, *M. fortuitum*, and nocardiae. A small group of nonphotochromogenic types exhibited great resistance to fading, and these strains differed from the other Battey strains in several other properties.

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MEASUREMENTS OF THE ACTIVITY OF AMBULATORY HOSPITALIZED TUBERCULOUS PATIENTS AND THE EFFECTS OF A TRANQUILIZING AGENT

Chemotherapy has resulted in liberalization of the amount of activity prescribed for patients with active tuberculosis. The ideal degree of permissible ambulation is yet to be determined. While prolonged bed rest is no longer deemed necessary in every case, few advocate unlimited activity throughout the period of therapy. Patient recalcitrance, economic and social pressures, and clinical experience influence the phthisiologist's recommendations. Thus, most physicians, although possessing a healthy respect for tuberculosis, are continuously extending the activity sphere of patients with this disease. As a rule, they are pleasantly surprised with their results. This has been the experience of the present writers. However, two questions remain unanswered. Exactly how active is a patient with tuberculosis who has been placed on modified bed rest? Can tranquilizers satisfactorily reduce physical activity when such a result is desired? To answer these questions, a study was initiated.

All patients on this service are required to observe bed rest for a total of two and one-half hours during morning and afternoon rest periods. Ward lights are extinguished at 9:30 p.m., and most patients awaken by 7:00 a.m. the following morning. During the remainder of the day (twelve hours), patients with tubercle bacilli in the sputum are restricted to the ward or the adjoining recreation room. No patient is farther than 15 yards from the lavatory or the recreation area. Patients with noninfectious sputum are permitted to go to the canteen, to the yard, or to entertainment programs on the hospital grounds. None of these are more than 150 linear yards from the bedside. All patients are particularly cautioned against overactivity during the first weeks and months of their hospitalization and later, after reversal of infectiousness and roentgenographic improvement, as indicated.

Ten patients were chosen to form the study group. Initially it was planned to study 5 patients with infectious and 5 with noninfectious sputum. However, since the observation period extended for three months, only 3 noninfectious patients were found who would not, on the basis of past behavior, request passes or leave the hospital during the study period. The only basis for selection of patients was the probability of continuing availability during a three-month period. Five patients were recently hospitalized and had tubercle bacilli in the sputum. The remaining 5 were inpatients with chronic pulmonary tuberculosis, 2 of whom had infectious sputum throughout the period of observation.

Each patient was provided with a commercially available pedometer, which was attached to the hospital garb, worn at all times, and read at weekly intervals. The nursing and professional staff, having been apprised of the problem, checked the patients many times each day to ensure continuous wearing of the device. No difficulty was encountered with this particular aspect of the study. Pedometers were worn for twelve weeks.

The first third of this period served as a basic control period. During the next eight weeks, all patients received identical capsules containing 0.25 mg. of reserpine or an inert placebo twice each day. These were prepared in the hospital pharmacy prior to initiation of the study and were administered by the double blind method. All patients received the tranquilizer for the course of two or four weeks' duration, preceded and followed by periods of placebo administration.

All patients completed the preliminary control phase of the study. There was considerable variation in the number of miles walked per week. However, generally speaking, each patient was fairly constant in the measurable extent of his weekly activity. The median mileage for all patients was 9.5 (range 4.5 to 14.0), and the average weekly mileage was 9.7. There was no significant difference between mileage of the 5 new patients and the 5 "old" patients, i.e., approximately one-half mile per week, when computed as average and median mileage. Furthermore, the differences between the new and "old" patients who comprised the infectious sputum group were of the same order, i.e., less than 1 mile per week when similarly computed.

Two patients were lost from the study at the end of the basic control period: one because of acute appendicitis, the other because of leaving the hospital on his own sanction. Later, further attrition occurred because of unsanctioned discharge (one case) and recurrence of peptic ulcer (one case). Only 6 patients completed the eight-week treatment period. Two others completed four weeks each. Thus, the treatment period may be said to have consisted of fifty-six weeks of patient observation. More than half of this period (thirty weeks) was during reserpine administration. During the remaining twenty-six weeks, the patients were given a placebo. For 5 patients there were two two-week periods of reserpine therapy, separated by a two-week period of placebo ad-

ministration. An additional patient had a single two-week period of observation while receiving the tranquilizer and prior to withdrawal from the study. Two of the patients were observed for a single four-week period of continuous reserpine administration in addition to a similar period of placebo administration.

During the period of placebo administration, the number of miles walked per week per patient averaged 11.6 (median 12.0) with a range of 0.5 to 19.25 miles. During reserpine therapy, the average was 11.5 (median 12.0) with a range of 6 to 19 miles.

Review of the individual records of activity revealed several interesting findings. One patient exhibited a sustained increase in activity of approximately 25 per cent (3 miles per week) during the period of administration of reserpine. There were 4 patients who, failed to evince decreased motor activity during or following administration of the ataractic although 2 of these did exhibit increase in ambulation throughout the placebo and drug period as compared to their control periods (15 and 120 per cent, respectively). Three patients engaged in decreased activity during the period of reserpine administration. In one of these, ambulation decreased throughout the four weeks of sedation and the following week of placebo therapy from an average of 15 to 10 miles in spite of over-all increased activity throughout this period as compared with the base control period. In the second patient, although ambulation was generally decreased during the treatment and placebo period, administration of the tranquilizer was associated with a further decrease in activity from an average of 8 to slightly less than 4 miles per week. The last patient, although much more active subsequent to the basic control period, showed a steady decline in activity from an average of 16 to 8 miles per week during the two weeks of reserpine therapy and the three succeeding weeks of placebo administration. This was followed by a 50 per cent increase in activity during the last week of placebo therapy, which increase was sustained during the next two weeks of observation when the ataractic was being given.

* * *

Because of the difficulties previously mentioned, the number of patients studied was small. Not only was the dose of reserpine moderate (0.5 mg. per day), but limitations of time precluded evaluation of the reported carry-over effect of the tranquilizer after discontinuation of administration. Subclinical psychic causes for increased or decreased ambulation could not be noted. Finally, as expected, ambulation increased with duration of institutionalization. For all of these reasons, it is impossible to attach real significance to the anti-ambulatory effect of the drug tested. It was a clinical impression—but only an impression—that at least 2, and possibly 3, of the patients did exhibit the desired response. In short, no conclusions can be drawn from this aspect of the investigation.

* * *

Of considerable significance was the actual measured activity of patients hospitalized on the modified bed-rest program utilized in this facility. This is more striking when it is recalled that the values obtained are minimal values. Sporadic checks of the professional staff, using the same pedometers, indicated that the ward physicians and nurses usually walked only 2.5 to 3.5 miles during an eight-hour working day, or 3.5 to 5.0 miles during a twenty-four-hour day. Yet some of these patients on "restricted activity" walked 16 to 19 miles per week. This was an unexpected finding and suggests re-evaluation of some current concepts.

In summary: The ambulatory activity of 10 patients, hospitalized with active pulmonary tuberculosis, was measured with pedometers. Administration of a tranquilizing agent was associated with a moderate decrease in activity in 3 of the patients. All patients were noted to be more active than implied by a modified bed-rest program. On occasion the amount of measured activity of the patients exceeded that of the professional staff.

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THE BEST DOCTOR IN THE HOSPITAL

Colonel Robert B. Skinner[1] recently wrote:

> The average patient does a good job of sizing you up. . . . The patient quickly senses the presence of interest among hospital personnel. . . . Too often time seems inadequate to allow for an explanation to the patient. They may not be told promptly, if at all, the rationale and the results of diagnostic procedures.

Colonel Skinner spoke about one patient who complained that in seventeen days of hospitalization nothing had been done for him. The Colonel was able to change this patient's attitude toward his treatment from skepticism to appreciation by taking a few minutes to explain his treatment.

As part of a large pilot study on hospital adjustment, known as a Cooperative Psychological Research Study in Tuberculosis, which is being conducted in nineteen Veterans Administration hospitals, 50 patients were given a battery of psychologic tests and filled out a special adjustment form. Likewise, various hospital personnel, including the ward physicians, were asked to fill out short forms devised to indicate the patient's hospital adjustment. All forms concerning the same patient had the same code number, but no names were put on any of the forms. This report will be limited to some of the items on the physician's form and to the items on the patient's form which concern the ward physician.

Sizing-up the ward physician: How the 50 patients answering one of the items on the patient's form "sized-up" their ward physician is shown in table 1. One patient was unwilling to commit himself and merely put a question mark on this item. Ten of the patients thought that their doctor was the best in the hospital. Eight thought their doctor was not as good as some. The rest, 31, said that their doctor was as good as the next. Offhand, this looks like a fairly normal distribution and hardly worthy of further comment. However, when these data are broken down by wards, there is a marked difference between the 67 per cent of patients in Dr. Z's ward, who consider him the best doctor in the hospital, and the 9 to 10 per cent of the patients in each of the other two wards, who consider their doctor the best. The numbers are small, however, so it might be well to look at some of the other items.

What does Dr. Z do, or what impression does Dr. Z make on his patients, that the majority consider him the best ward physician in the hospital? As shown in table 2, Dr. Z consistently scores higher than the other two ward physicians by spending much time on the ward, talking to the patients about their tuberculous condition as fully as they think necessary, and showing that he likes the patients. No patient accuses him of spending very little time on the ward. None of his patients checked "not very often" for "Our doctor talks to us about our TB condition," although this was marked by 19 per cent of Dr. X's patients and 55 per cent of Dr. Y's patients. This, despite the fact that Dr. Z has had a very busy ward. His patients have sometimes told the psychologist that they hated to bother him by asking questions because they knew he was very busy, but they knew that he would take time to explain their condition and show them their X-rays if they asked. Although Dr. Z's patients are more satisfied with the time devoted to them and the explanations he gives his patients regarding their condition, he does not feel that they make excessive demands upon him. As may be seen in table 3, item *A*, he marked 56 per cent of his patients as "Seldom asks for explanations," while the other two ward physicians marked this response for only 10 and 30 per cent, respectively, of their patients.

The ward physician considered the best in the hospital by most of his patients seemed to the majority of his patients (78 per cent) to show frequently that he liked the patients on his ward (table 2; item *C*). Quite likely it is not that he actually liked or cared for his patients so much more than the other physicians, but he was able to get across to them better than Dr. Y, at least, that he liked them; that taking care of them was not just a job, not just a way of making a living. In fact, he was the only physician (table 3, item *B*) who admitted that one patient was a "less likeable person than other patients."

Contrasting best with not as good: So far the primary concern has been what patients from Dr. Z's ward said about him. It will be recalled (table 1) that 9 and 10 per cent, respectively, of the patients from the other two wards considered their ward physician the best in the hospital. In table 4, all of the patients are compared who considered their ward physician the best in the hospital with those who considered their ward physician not as good as some. It was found that 80 to 90 per cent of those who considered their ward physician the best in the hospital credited him with spending much time on the ward, talking to them about their condition as much as they considered necessary, and often showing that he liked the patients

[1] SKINNER, R. B.: Med. Technicians Bull., 1958, *9*, 19.

on his ward. In contrast, only 25 to 50 per cent of the patients who considered their ward physician as not as good as some described their ward physician the same way.

Surgery items: Both the patient's form and the physician's form contained items regarding surgery, but less reliance can be placed upon the results for this hospital alone because surgery has not been recommended for all of the patients. Some had had it recommended before the patient was transferred to this hospital or before he was transferred to his present ward. Consequently, while 25 patients answered the item regarding the explanation given him concerning surgery, the ward physicians checked the corresponding item indicating the patient's reaction to the recommendation for only 11 patients, too few for meaningful analysis.

Final disposition of all of the patients in this study is, of course, incomplete. In view of the fact, however, that patients frequently threaten to leave without sanction or give as a reason for leaving in this way that they have not been told anything about their condition, a preliminary follow-up three months after the last of this data was collected was made to see what kind of discharges the patients received. Of the 8 patients checking, "If surgery has been recommended, he explained it to me *completely*," 5 have been given "good discharges" and the other 3 are still in the hospital. On the contrary, of the 5 who checked, "If surgery has been recommended, he explained it *hardly at all*," 2 have left without sanction and the other 3 patients are still in the hospital. Further investigation is indicated to determine the effect of explanations satisfactory to the patient on his acceptance of treatment, including surgery, and on the type of discharge he receives.

TABLE 1

THE DOCTOR ON OUR WARD IS:

	Number	Per Cent for Each Ward Physician*		
		Dr. X	Dr. Y	Dr. Z
a. The best in the hospital	10	9	10	67
b. As good as the next	31	81	60	22
c. Not as good as some	8	5	30	11
?	1	5	0	0
Total	50	100	100	100

* For purposes of comparison, the per cent of patients from each ward marking each response is given. There were 21 patients from Dr. X's ward, 20 from Dr. Y's ward, and 9 from Dr. Z's ward.

TABLE 2

SOME ITEMS ON PATIENT'S FORM RELATING TO WARD PHYSICIAN

	Number	Per Cent for Each Ward Physician*		
		Dr. X	Dr. Y	Dr. Z
A. Our doctor spends				
1. Much time on the ward	28	57	40	89
2. A fair amount of time on the ward	17	38	40	11
3. Very little time on the ward	5	10	20	0
B. Our doctor talks to us about our TB condition				
1. As much as we need	23	38	35	89
2. Sometimes	12	38	10	11
3. Not very often	15	19	55	0
C. Our doctor shows that he likes the patients on the ward				
1. Often	29	76	30	78
2. Occasionally	21	24	70	22
3. Never	0	0	0	0

* Per cent of times the patients from a particular physician's ward marked each response. There were 21 patients from Dr. X's ward, 20 from Dr. Y's ward and 9 from Dr. Z's ward.

The patients frequently reveal to the psychologist their anxiety concerning lack of information regarding their disease. David Lord,[2] writing about communication between physician and patient in a tuberculosis hospital said:

" . . . one way in which a man handles his travail is to get some sort of intellectual grasp of the situation, to try symbolically to control it by "knowing" about it. This is the right of any patient. This right should not be ignored or denied."

In this hospital, this right has not been ignored. From the physicians' point of view, the patients have had their tuberculous condition explained to them repeatedly. Stratton[3] found, however, upon administering a vocabulary test consisting of words used constantly by physicians in a tuberculosis hospital: ". . .that a large number of the concepts used by the staff of a tuberculosis hospital

[2] LORD, D.: Newsletter for Psychologists in Tuberculosis, 1957, *4*, 84.

[3] STRATTON, A. J., JR.: Newsletter for Psychologists in Tuberculosis, 1957, *4*, 38.

TABLE 3

SOME ITEMS FROM PHYSICIAN'S FORM

	Number	Per Cent for Each Ward Physician*		
		Dr. X	Dr. Y	Dr. Z
A. Patient asks for explanation as to his TB condition				
1. More than most patients	14	38	20	22
2. An average amount	23	52	50	22
3. Seldom asks for explanation	13	10	30	56
B. Patient compared to other patients				
1. A more likeable person	24	52	45	44
2. About average	25	48	55	44
3. Less likeable than other patients	1	0	0	11
C. The patient				
1. Follows advice readily	23	29	65	44
2. Follows advice as much as the next patient	16	43	15	44
3. Is somewhat resistive to medical advice	11	29	20	11
4. Is very resistive to medical advice	0	0	0	0

* Per cent of times each physician marked certain responses. Dr. X marked for 21 patients, Dr. Y for 20 patients, and Dr. Z for 9 patients.

TABLE 4

COMPARISON OF BEST DOCTOR WITH DOCTOR NOT AS GOOD AS SOME

	Per Cent Best	Per Cent Not as Good
Spends much time on ward	90	25
Talks to us about our TB condition as much as we need	90	37
Often shows that he likes the patients on his ward	80	50

are poorly understood by the patients." This may account in part for the apparent discrepancies between statements of patients and ward physicians regarding explanations given to patients. What may be an explanation to the physician may be completely incomprehensible to the patient. This may help to explain why less than 50 per cent of the patients indicated that "Our doctor talks to us about our TB condition *as much as we need*" (table 2, item *B*). Maybe he talks to them about their condition, but not always in their language.

Approximately a week after admission, the patient is brought into the therapy board for a few minutes. He is briefed on his condition, shown his roentgenograms, and frequently warned of the dangers of an unsanctioned discharge. He is given an opportunity to ask questions, but usually is unable to formulate any except perhaps, "How long will I have to stay?" What further explanation the patient receives after the initial visit to the therapy board depends on the ward physician, for it is the policy at this hospital that only the ward physician shall give the patient any information regarding his condition. Dr. Z's policy is to grant a patient an interview whenever he asks for one, on the basis that maybe one of four requests may be important—in the judgment of the physician. Dr. Y's policy is to withhold all information regarding laboratory work, such as sputum tests and roentgenograms, until after the patient "is boarded" so that he can be given a complete picture at one time. Laboratory work such as sputum cultures is frequently done at least once a month, but patients are routinely "boarded" (without the patient being present) only every three or four months. Dr. X is reluctant to let his patients know when their cases have been considered by the medical board. He thinks that, if they know their sputum has come back negative for tubercle bacilli or that their roentgenograms show improvement, they will make their own interpretations and think that they are well enough for a pass or a discharge. Patients who have improved clinically under chemotherapy tend to reject violently the physician's statement that they still have a cavity when he waits until they ask for a pass to give them this information. It is not surprising, therefore, that Dr. X finds 29 per cent of his patients somewhat resistive to medical advice, while Dr. Z finds only 11 per cent of his patients somewhat resistive (table 3, item *C*).

Anyone who has ever worked in a tuberculosis hospital knows that three months is a short time for treatment of the disease and that marked changes are rarely seen on a roentgenogram in less than three months. But to the patient anxious to know about his progress, three months can be a seemingly endless procession of days, hours, minutes, and seconds. Perhaps no one who has not been isolated and suspected of having tuberculosis can appreciate how the patients feel. Perhaps, however, those who have been in a combat area without adequate intelligence to know the

strength and location of the enemy can appreciate the anxiety caused by just *not knowing*. Perhaps the corpsman who has crawled under enemy fire for thirty minutes to rescue a wounded marine can appreciate the relativity of time.

In summary: As part of a much larger Cooperative Psychological Research Study in Tuberculosis, 50 patients in a Veterans Administration tuberculosis hospital were given a patient's form to check, including several items relating to their ward physician. The ward physicians were also given a short form to check regarding these patients. The ward physician whose patients most frequently considered him the best doctor in the hospital was described more frequently as spending much time on the ward, often showing that he liked his patients, and talking to them about their condition as much as they thought necessary. In turn, the physician most frequently rated the best by his patients thought that most of his patients in this study seldom asked for explanations of their condition and accepted medical advice.

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November 4, 1958

TUBERCULOSIS CASE FINDING IN PSYCHIATRIC HOSPITALS

A Discussion of the Resurvey Interval

Although the literature on the control of tuberculosis in psychiatric hospitals is rather sparse, it should be generally agreed today that this disease has no characteristics which are peculiar to the patient population of mental disease institutions. As Katz and associates[1] and Myers[2] have emphasized, there is nothing about tuberculosis in psychiatric patients that cannot be explained by "epidemiologic factors within the concept of tuberculosis as an infectious disease"[1] and there is no real evidence to suggest that patients with mental disease have a constitutional predisposition to tuberculosis. Where there is a well-organized program for the control of tuberculosis based on the principles of case finding, isolation, and therapy of active disease, the incidence of new cases can be shown to approach the level in the general population even though the original prevalence of tuberculosis was much higher in the institution than in the general population. Therefore, ordinary methods of tuberculosis control are effective in the institutional environs and perhaps more effective than in the general population because the institutional population is more or less captive.

However there is one question on whose answer agreement is not complete: how often should chest roentgenographic resurveys be done among inmates of psychiatric hospitals? This question is of particular importance today because of the fears which have arisen concerning excess irradiation, whether these fears be valid or not. The most common recommendation is that the interval between resurveys be one year. This is the recommendation of the American Trudeau Society and of the American College of Chest Physicians. Yet there is no evidence that a two-year, three-year, or four-year interval is unsatisfactory.

The decision as to what interval is indicated must be based on a number of factors, the most obvious of which seem to be: (*1*) the type of patient population: if there is a high percentage of persons in whom tuberculosis may progress rapidly, such as non-whites, then the interval should be short; (*2*) the degree of control of tuberculosis: as control improves, the yield of resurveys diminishes and levels off so that the interval may be lengthened; and (*3*) the cost in relation to the yield: as the yield diminishes, the cost of resurveys at short intervals becomes prohibitive.

The accepted index to the control of tuberculosis today is the incidence of the disease or the number of new cases per unit population per year. The proper figure can be derived only after one or more resurveys of a given population because the results of the first survey represent the prevalence of tuberculosis, i.e., the number of cases which have accumulated over a period of years. (As used herein, a new case of tuberculosis refers to a case of active disease as defined by the 1955 National Tuberculosis Association classification.)

From the literature one may obtain some incidence figures arranged by duration of the resurvey interval (table 1). An uncritical perusal of the figures might suggest that, when the resurvey interval is five years, the new case rate is much greater than it is when the interval is four years or less; and that there is little difference in the new case rate when the resurvey interval varies between one and four years. However, there are a number of recognizable factors which make it dangerous to draw such conclusions, factors which indicate that the data from which the various new case rates were derived come from universes which are not strictly comparable. Factors to be considered are as follows:

The year for which the figure is given varies from 1946 to 1956. During that ten-year period there was no remarkable drop in the new case rate in the general population of Philadelphia, the large city near Norristown State Hospital. The Philadelphia new case rate was 1.1 per 1,000 per year in 1946 and 1.0 in 1956. However, in some other areas of the United States there was a larger decrease. A drop in the incidence of tuberculosis during the last decade is due to a number of factors, among which are the natural decline of the disease, improved control, and the advent of effective antimicrobial therapy. The figures in table 1 can be rearranged in relation to time and a correlation found with the highest new case rate in 1946 and the lowest in 1956. It is not certain how much of the difference between the highest and lowest new case rates could be due to these factors represented by time, especially in view of the fact that changes in the degree of case reporting undoubtedly occur from time to time.

Different population segments are involved because of variation in place. It would not be expected that the inmates of California institutions would have exactly the same characteristics as those of New York state institutions. In addition figures for the Veterans Administration hospitals

[1] Katz, J., *et al.*: Am. Rev. Tubere., 1954, *70*, 32.
[2] Myers, J. A.: Dis. Chest, 1957, *31*, 384.

TABLE 1

NEW CASE RATES IN TUBERCULOSIS IN PSYCHIATRIC HOSPITALS BY RESURVEY INTERVAL

Resurvey Interval in Years	Source	Year of Last Survey	Number of Previous Surveys	Approximate Population	New Case Rate	Bacteriologic Studies Done	Cases Discovered by Symptoms Included
					no./1,000/yr.		
1	Oechsli: California state hospitals[3]	1950	4	29,000	1.8	No	Not mentioned
	Clark and Weber: Ontario psychiatric hospitals[4]	1950	13	18,000	2.2	Yes	Not mentioned
2	Weiss*: Norristown State Hospital	1956	2	1,700	1.7	Yes	Yes
4	Katz *et al.*: N. Y. state psychiatric hospitals[1]	1952	3	Not given	2.2	Yes	No
5	Pleasure: Central Islip State Hospital, New York[5]	1946	1	7,000	7.6	Yes	Yes

* Data presented in this paper.

and the Norristown State Hospital refer only to male patients. (The figure for female inmates at the Norristown State Hospital is not yet available.) The incidence of tuberculosis varies somewhat with sex. It also varies with age and race. At the Norristown State Hospital the inmates are predominantly old white people. The prevalence—and therefore the incidence—of tuberculosis is known to be higher in older persons, non-whites, and males.

The number of previous surveys is an important factor in the new case rate because, as Oechsli[3] and Clark and Weber[4] have shown, there is a rapid diminution in the new case rate with the first few surveys and then a leveling out of the figures. The plateau probably is reached after a different number of resurveys in different institutions, depending upon many other factors involved in the policies applied to the detailed conduct of the control program, as mentioned below. However, the plateau should be reached after the first few surveys in careful programs.

The conduct of the control program varies considerably in different institutions. For example, the figure for California institutions is based on roentgenographic impressions alone without bacteriologic studies. This was also true at Norristown State Hospital during the period of the first two surveys and, therefore, the figure in the present study has been limited to the two-year period after the second survey. The lack of bacteriologic confirmation of the diagnosis of tuberculosis introduces an unknown degree of error which is lessened but not eliminated by attempts at bacteriologic studies.

Even in institutions where bacteriologic studies are attempted (and the term "attempted" is used advisedly because it is often impossible to obtain satisfactory twenty-four-hour sputum specimens from psychotic patients), there are many patients who are treated as tuberculous without benefit of bacteriologic confirmation. For example, in the third survey at the Norristown State Hospital, 20 male inmates were isolated for study as a result of a roentgenographic reading of active tuberculosis or advanced questionably active tuberculosis. All but 3 of these patients had had previously known abnormal films. The disease in 11 was minimal, 8 had moderately advanced disease, and the disease in one was far advanced. After study, the disease in 4 patients was considered inactive or nontuberculous. In the remaining 16 who were treated as tuberculous, sputum was unobtainable in 4, positive for tubercle bacilli in 7, and negative in 5. It is possible that a few of these 9 patients without bacteriologic confirmation may have had disease other than tuberculosis, e.g., coccidioidomycosis or histoplasmosis.

The type of survey-discovered case which is isolated varies with the facilities available, as mentioned in different reports. At the Norristown State Hospital, isolation is used for all patients whose survey films are read as active tuberculosis and for those whose films are read as questionably active, if the extent of disease is moderately or far

[3] OECHSLI, W. R.: Pub. Health Rep., 1954, *69*, 560.

[4] CLARK, E. A., AND WEBER, J. J.: Ment. Hosp., 1952, *3*, 4.

advanced. Thus, patients with minimal questionably active disease and all patients whose films indicate active tuberculosis are examined roentgenographically at three- to twelve-month intervals and isolated when there is roentgenographic evidence of activity. Such a policy provides a small loophole for spread of infection from patients who are actually contagious though not recognized as such from the roentgenogram alone. In practice it is difficult to "plug" this loophole.

At the Norristown State Hospital complete facilities for control of tuberculosis were obtained by 1954, including a part-time attending physician in pulmonary disease, stationary photofluorographic equipment, a planigraph, a bacteriology technician, and an attending surgeon. The present plan for the control of tuberculosis includes:

1. Chest roentgenographic surveys of new admissions and readmissions on 14- by 17-inch films; of new employees on photofluorograms
2. Chest roentgenographic resurveys of inmates on photofluorograms every two years; of employees on photofluorograms every year
3. Isolation, study, and treatment (if indicated) of all patients with active and advanced questionably active disease
4. Periodic roentgenographic follow-up study of patients with suspect, inactive, and minimal questionably active disease at suitable intervals
5. Reporting of all cases of tuberculosis to the division of tuberculosis control in the state health department.

Photofluorograms are used purely for screening purposes. Since admissions have a high prevalence of abnormal films, 14- by 17-inch chest films were considered preferable to photofluorograms.

A tuberculin testing program has not been inaugurated because a tuberculin survey of new admissions has shown that more than 90 per cent have positive reactions to first- or second-strength PPD-S (1957).

Calculation of the new case rate varies in different reports. Cases of active tuberculosis discovered as a result of the development of symptoms in the interval between roentgenographic surveys are not mentioned by Oechsli[3] or Clark and Weber[4]; are mentioned, but not included, by Katz and associates[1]; and are included by the present writer and by Pleasure.[5] The cases discovered because of symptoms are important because they occur even with a short resurvey interval.

In the present writer's experience, a patient may go from a normal roentgenogram to symptoms of advanced tuberculosis in as short a period as seven months. Therefore, a resurvey interval as short as one year would fail to discover an occasional case. Since cases detected through symptoms are also new cases of tuberculosis, they must be included in the calculation of the new case rate. For example, at the Norristown State Hospital, the third survey of male inmates uncovered 3 new cases of minimal active tuberculosis; but during the second-year interval between the second and third surveys, 2 additional cases of active tuberculosis were uncovered as a result of symptoms. One patient developed an acute tuberculous pleural effusion, proved by needle biopsy of the parietal pleura; and another patient developed a minimal pulmonary lesion to which attention was attracted by a superimposed pyogenic pneumonia (the tuberculosis was confirmed by a sputum culture positive for tubercle bacilli). Thus, in approximately 1,700 male inmates, 5 new cases were found in a period of two years, making a new case rate of 1.7 per 1,000 per year.

From all that has been said above, it is obvious that incidence figures for tuberculosis in psychiatric hospitals are not drawn from comparable data. Despite this, it is remarkable that the new case rates reported since 1950 from four groups of institutions (*see* table 1) following two or more resurveys are all of the same order of magnitude, about 2 per 1,000 per year, despite the fact that the resurvey interval varied from one to four years. While these incidence rates are significant, all of the 5 cases discovered by survey or symptoms in the Norristown State Hospital were still minimal despite the fact that the resurvey interval was two years. A shorter resurvey interval could hardly have accomplished more.

In contrast to the small number of new cases[6] which occurred at Norristown State Hospital between the second and third surveys of male inmates, there were 21 previously known cases of tuberculosis that became active. A reactivation rate can therefore be calculated for known cases of tuberculosis per 1,000 male inmates per year: 6.2, which is more than four times the figure for the new case rate. A very similar figure is given by Oechsli, Kupka, and Bush.[6] Most of the reactivated cases were picked up by the third survey, but a good follow-up system is now in effect so that it is no longer necessary to depend on surveys to find these persons. Obviously it is important to "keep tabs on" the known cases of tuberculosis which are uncovered by each survey

[5] Pleasure, H.: Psychoanalyt. Quart., 1948, *22*, 445.

[6] Oechsli, W. R., *et al.*: Pub. Health Rep., 1955, *70*, 1194.

because they constitute the largest source of active disease with the passage of time, once some control of tuberculosis has been established.

In summary: It seems logical to conclude that no general statement can be made of what the resurvey interval should be for captive populations. If the interval is too short, the cost is not justified by the yield. If the interval is too long, control of tuberculosis may be inadequate. Once good control is achieved, the optimal interval must be determined for each institution by the yield in relation to the effort required. It appears that a two- to four-year interval is reasonable provided a good over-all control program is in use.

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November 14, 1958

[7] The writer is grateful to Dr. Katharine R. Boucot, Professor of Preventive Medicine at the Woman's Medical College of Pennsylvania, for review and constructive criticism of this paper.

THE USE OF ENZYMES TO AID FILTRATION OF OROPHARYNGEAL WASHES THROUGH MEMBRANE FILTER[1, 2]

Saline rinses of the oropharyngeal region have been used in studies on the retention of bacteria in the upper respiratory tract,[3, 4] and in the diagnosis of pulmonary tuberculosis[5] and of anthrax infection.[6] Normally these rinses, containing saliva, cannot be filtered readily through the membrane filter, and a suitable aid to filtration is required. In the studies cited above, use was made of the findings reported here, namely, that the addition of several crude enzymes to saline rinses markedly increased ease of filtration.

Sterile solutions of various enzymes were added to oropharyngeal saline rinses (mixed samples from 8 to 15 men) and were filtered after incubation for twenty to forty minutes at 37°C. Suitability for the purpose was judged on the basis of the time required for filtration of a standard volume of a treated sample through a membrane filter.[7] None of the enzymes tested decreased the number of *Serratia marcescens* cells added to saline rinses prior to enzyme treatment. The following enzymes[8] were found to be effective: mycozyme, α-amylase, β-amylase, hyaluronidase, and glucuronidase. Amylopsin, protease, and trypsin had little or no effect on filtration times. In a number of instances mere incubation without added enzymes reduced filtration times. Also, in a significant number of trials, Triton X-100[9] at 0.04 per cent was found to be effective. However, the best combination appeared to be 5 ml. of 20 per cent solution of mycozyme plus 5 ml. of a 5 per cent solution of α-amylase per 25 to 50 ml. of saline rinse with incubation at 37°C. for thirty minutes prior to filtration. Solutions of these enzymes could be combined and held for several months in the refrigerator, or could be lyophilized[10] and held at room temperature under vacuum for three years or more.

Dubos[11] and Rawlins [12] have reported on the use of crude pancreatic enzymes for the liquefaction of sputum prior to bacteriologic examination. These enzymes, as well as those used above, contain polysaccharide hydrolases and it would appear that their effectiveness for the purpose used is dependent upon their ability to hydrolyze salivary mucopolysaccharide.

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December 2, 1958

[1] This work was sponsored by the Bureau of Medicine and Surgery, U. S. Navy, under a contract between the Office of Naval Research and the Regents of the University of California.

[2] Opinions expressed in this report are not to be construed as reflecting the views of the Navy Department or of the naval service at large (Article 1252, U. S. Navy Regulations, 1948). Reproduction in whole or part is permitted for any purpose of the United States Government.

[3] Ostrom, C. A., *et al.*: Unpublished data, 1957.

[4] Ostrom, C. A., *et al.*: J. Infect. Dis., 1958, *103*, 251.

[5] Rogers, D. E., *et al.*: Am. Rev. Tuberc., 1955, *71*, 371.

[6] Carr, E. A., and Rew, R. R.: J. Infect. Dis., 1957, *100*, 169.

[7] Manufactured by Millipore Filter Corp., Watertown, Massachusetts.

[8] Product of General Biochemicals, Inc., Chagrin Falls, Ohio.

[9] Product of Rohm and Hass Co., Philadelphia, Pennsylvania.

[10] The writers wish to thank Dr. R. J. Heckly and Mr. M. M. Klumpp for carrying out the lyophilizations.

[11] Dubos, R. J.: Tr. Ninth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1950, p. 229.

[12] Rawlins, G. A.: Lancet, 1953, *2*, 538.

HISTOPLASMIN SENSITIVITY IN ALASKAN NATIVES

Recent reviews of the geographic distribution of histoplasmin sensitivity reveal that there are still many areas of the world for which there is little or no information on this subject.[1, 2] One of them is Alaska, where the results of histoplasmin testing have been reported only from the Pribiloff Islands[3] and the southeastern panhandle.[4] The purpose of this Note is to add a small bit to the knowledge of histoplasmin sensitivity in Alaska.

TABLE 1

SIZE OF REACTIONS TO HISTOPLASMIN AMONG ALASKAN NATIVES

Induration in millimeters	Total	0	2	3	4	5	6	21
Number of persons	509	488	8	4	6	1	1	1

During April, 1957, a tuberculin-testing survey was conducted by the Tuberculosis Program of the Public Health Service in cooperation with the Arctic Health Research Center among villages along the lower Yukon River and on the southeastern shore of Norton Sound. Three-fourths of the population participated in the survey. In Marshall, Piamute, Russian Mission, and St. Marys (Andreafski), and for part of the participants in Unalakleet, histoplasmin (0.1 ml. of a 1:50 dilution of H-42) was given in one forearm and tuberculin in the other. The tests were given by three experienced nurses of the Tuberculosis Program and were read forty-eight hours later by the same personnel.

The results are shown in table 1. All of the 509 persons tested with histoplasmin were Eskimos except for 15 Indians from Piamute. Among the 488 persons with no induration, 34 had some erythema. There were no significant differences in the distribution of reactions by race, sex, or village. Seven per cent of those more than fifteen years of age and 3 per cent of those less than this age showed some induration.

The person for whom 21 mm. of induration was recorded was a 16-year-old boy from Unalakleet whose first five years had been spent in Nome. In this instance, a recording error cannot be excluded since his tuberculin reaction was recorded as showing no induration, whereas, among persons of his age, 95 per cent had tuberculin reactions larger than 5 mm.

The distribution of reactions 2 to 6 mm. in diameter suggests that they may have resulted from needle trauma or some substance in the buffering solution. There is no indication of false positive reactions resulting from a primary irritant effect of the histoplasmin preparation. Nor is there any evidence that this population has been infected with other fungi, such as coccidioides, which may cause cross-reactions to histoplasmin.[5]

In summary: The results of histoplasmin testing of 509 natives in five villages near the mouth of the Yukon River in Alaska are reported. Three persons, or 0.6 per cent of the tested population, had reactions of 5 or more mm. of induration. However, there is reason to believe that there is no specific histoplasmin sensitivity in this population.

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December 8, 1958

[1] MOCHI, A., AND EDWARDS, P. Q.: Bull. World Health Org., 1952, *5*, 259.

[2] EDWARDS, P. Q., AND KIAER, J. H.: Am. J. Trop. Med., 1956, *5*, 235.

[3] SEXTON, R. L., *et al.*: J. Allergy, 1949, *20*, 133.

[4] ARONSON, J. D.: Personal communication, quoted in footnote [1].

[5] PALMER, C. E., *et al.*: Am. J. Hyg., 1957, *66*, 196.

Correspondence

VOCATIONAL REHABILITATION IN TUBERCULOSIS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

I would appreciate it if you would consider my following comments on the article by Drs. Carr and Bridge concerning the "Vocational Rehabilitation in Tuberculosis" which appeared in the October, 1958, issue of the REVIEW.

Rehabilitation has been an effective part of the tuberculosis control program because it has been practiced and applied in the broadest sense. It has been an individualized process with emphasis on the patient rather than the disease.

The TB patient or any other patient cannot be treated in segments. Vocational planning cannot be isolated from his medical, psychologic, or social environment. Therefore, to conclude that fewer patients need vocational rehabilitation for medical reasons is to tell only part of the story, and that part, out of context. The need continues to be for comprehensive services for the total person.

It is agreed that the time has come for a "reconsideration" based on the developments of the last decade as outlined by Drs. Carr and Bridge. This reconsideration should be constructive. Let us reappraise our present-day facilities and techniques in relation to the present-day TB patient who is a candidate for rehabilitation. He is the multi-problem patient who, ten years ago, would not have survived to reach rehabilitation. Such patients may be fewer in number, but more specialized services will be needed to prevent relapse; to bring about rehabilitation for productive living.

Any funds, government or otherwise, expended for the provision of comprehensive rehabilitation services for this surviving group is money well spent.

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December 11, 1958

DIFFUSE INTERSTITIAL PULMONARY FIBROSIS AND HYPERTROPHIC PULMONARY OSTEOARTHROPATHY

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

Three cases of diffuse interstitial pulmonary fibrosis (Hamman-Rich syndrome) were reported by me in the October, 1958, issue of THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES. The presence of hypertrophic pulmonary osteoarthropathy was noted but was not stressed in the third case report. At the patient's first hospital admission in 1947, symptomatic and roentgenographic evidence of hypertrophic pulmonary osteoarthropathy was demonstrated. Intermittently, throughout the next several years, he had considerable discomfort especially in the knee and ankle joints; however, on his final admission, and for several months preceding, he was free of joint pain.

After reviewing reports of about 40 cases cited in the bibliography of my article, I was unable to find another instance of diffuse interstitial pulmonary fibrosis coexisting with hypertrophic pulmonary osteoarthropathy. Although clubbing of the fingers in patients with diffuse interstitial pulmonary fibrosis was reported, the remaining three portions of the "tetrad": (*1*) periosteal new bone formation, especially at the ends of long bones; (*2*) pain and swelling of the joints; and (*3*) autonomic dysfunction, were not mentioned. In a search for sarcoid lesions as part of the differential diagnosis in their case, Tuft and Girsh (Am. J. M. Sc., 1958, *235*, 60) reported that the long bones appeared normal on roentgenograms and that the patient had no bone or joint pain. Moore and co-workers (A. M. A. Arch. Int. Med., 1957, *100*, 651) reported that their patient had recurrent pain in his right hip, but roentgenograms (including views of the hands) showed no abnormalities to suggest hypertrophic pulmonary osteoarthropathy.

In patients with the Hamman-Rich syndrome, hypertrophic pulmonary osteoarthropathy has not been reported previously, nor has the Hamman-Rich syndrome heretofore been included among the conditions associated with or producing hypertrophic pulmonary osteoarthropathy. The relationship is an interesting one which should be noted.

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December 17, 1958

ON THE FREQUENCY OF ACID-FAST CHROMOGENS IN THE GASTRIC JUICE OF NONTUBERCULOUS PATIENTS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

It has been reported that atypical, chromogenic variants of H37Rv may be produced by suitable

in vitro exposure of typical organisms to streptomycin and isoniazid over a period of many months (TARSHIS, M. S.: Am. Rev. Tubere., 1958, *78*, 921). This might provide an explanation for the observation that chromogenic, acid-fast organisms have been encountered with increasing frequency since the mid-1940's in tuberculosis sanatoriums. A particularly high incidence of skotochromogens has been reported in gastric aspirates obtained after a course of antituberculous chemotherapy, such organisms appearing after the disappearance of typical *M. tuberculosis* (KELTZ, H.: Tr. Sixteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1957, p. 290). The significance of this observation is limited by a lack of controlled studies. In order to determine the frequency of chromogenic acid-fast bacilli in patients free from active tuberculosis and who had never received antituberculous chemotherapy, we have cultured the basal gastric aspirate obtained in the course of routine gastric secretory tests at the University of Chicago Clinics.

A total of 400 patients have been studied. Steenken and Smith media were used throughout, and digestion was carried out with 3 per cent sodium hydroxide for one hour prior to concentration. In 175 cases, duplicate samples were digested at 37°C. and room temperature. The remaining 225 specimens were digested at 37°C. only. Acid-fast bacilli were cultured on two occasions. One was culturally typical of *M. tuberculosis*; this patient was found to have active pulmonary lesions which had not been previously suspected. The only other acid-fast bacillus cultured proved to be a skotochromogen, of lemon yellow color, which appeared on the media at six weeks. This patient was carefully examined and found free of pulmonary disease—three subsequent gastric aspirates in this subject were completely negative. Digestion at room temperature instead of at 37°C. greatly increased contamination with nonacid-fast organisms, but no acid-fast bacilli were cultured from these specimens.

The fact that chromogenic mycobacteria could be cultured from the gastric juice of only one of 400 nontuberculous patients suggests that these organisms are infrequent incidental saprophytes in the population as a whole. The frequent isolation of such organisms in tuberculosis sanatoriums would seem related in some way to tuberculous infection or to the chemotherapy thereof.

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December 19, 1958

A PLEA FOR A CLEARER DISTINCTION BETWEEN ALLERGIC GRANULOMATOSIS AND WEGENER'S GRANULOMATOSIS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

Dr. John Read,[1] in his article published in the September number of the REVIEW, quotes as belonging to the description of Wegener's granulomatosis articles which actually refer to allergic granulomatosis. Consequently he describes as belonging to Wegener's granulomatosis some features seen by Churg and Straus[2] and Ehrlich and Romanoff[3] in cases of allergic granulomatosis. On the other hand, he does not quote the articles that Churg and associates[4, 5] wrote on the subject of Wegener's granulomatosis.

In the recent literature on the subject, one notes *first* some unawareness of the existence of allergic granulomatosis and *second* some confusion between the latter and Wegener's granulomatosis. As an example of my first assertion, I quote a case report published by Zasly and Baum.[6] It was a case of allergic granulomatosis, fitting the excellent description given by Churg and Straus. I think allergic granulomatosis could be called "Churg and Straus disease," thus making an easier distinction between "Wegener's granulomatosis" and "Churg and Straus granulomatosis." The confusion between these two different diseases is bound to increase since some authors have started referring to Wegener's granulomatosis as "pathergic." Beside the fact that "pathergy" is a bad name from an immunologic point of view, pathergic and allergic granulomatosis are easily thought to be the same.

Hood and associates[7] described recently 5 cases of "Wegener's pathergic granulomatosis" which cannot be accepted as such. The first 4 are more likely cases of eosinophilic granuloma of the lung, similar to cases described by Lackey and coworkers[8] and by Mazzitello[9]; in the fifth case there was even a bone lesion.

Some degree of confusion is bound to occur among entities as eosinophilic granuloma of the

[1] READ, J.: Am. Rev. Tubere., 1958, *78*, 353.
[2] CHURG, J., AND STRAUS, L.: Am. J. Path., 1951, *27*, 277.
[3] EHRLICH, J. C., AND ROMANOFF, A.: A. M. A. Arch. Int. Med., 1951, *87*, 259.
[4] GOODMAN, G. C., AND CHURG, J.: A. M. A. Arch. Path., 1954, *58*, 533.
[5] FAHEY, I. L., *et al.*: Am. J. Med., 1954, *17*, 168.
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lung, allergic granuloma, and Wegener's granuloma, since in all three the presence of eosinophils is an important feature. I think the following criteria could be adopted: Eosinophilic granuloma of the lung is a reticulosis or histiocytosis without vascular participation but the inevitable vascular alterations within the lesion itself; allergic granuloma is a lesion accompanied by vascular alterations varying from a simple intimal thickening to acute necrotizing arteritis; in Wegener's granuloma we find a giant cell granuloma and necrosis in the wall of vessels within the lesion and in the wall of vessels far removed from the site of the parenchymal granulomas. In neither eosinophilic granuloma nor Wegener's granuloma do we find eosinophils in the blood; whereas high eosinophilic counts are an important feature in the blood and exudates of patients with allergic granulomatosis.

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December 29, 1958

Books

CHRONIC BRONCHITIS, EMPHYSEMA AND COR PULMONALE. By C. H. Stuart-Harris and T. Hanley. Pp. 235, The Williams and Wilkins Co., Baltimore, Maryland, 1957. Price, $8.50.

Here is a comprehensive look at the most common diseases of the lung causing shortness of breath, as seen in Great Britain. It is largely a report of ten years' work at the University of Sheffield. Through systematic studies of the natural history of chronic bronchitis, including not only hospital studies but also inquiries made in industrial and rural communities, the authors gained an extraordinary breadth of knowledge. They move confidently through the specialized domains of the pathologist, the microbiologist, the physiologist, and the epidemiologist, and discuss all findings from the common-sense point of view of the clinician. This is a pleasure to witness.

The book deals primarily with chronic bronchitis identified by the presence of chronic cough, sputum, and breathlessness. After dealing with clinical features, the authors discuss at considerable length the bacteriologic aspects and the role of viral infections. In a section on morbid anatomy, which draws heavily on the excellent work of Lynne Reid at the Brompton Hospital, they concluded that the pathologic changes "suggest a disease affecting the bronchioles much more than the bronchi." Since the bronchioles are considered the site of the most important pathology and bronchiolar narrowing, the cause of the shortness of breath, one wonders if the disease under consideration is not "chronic bronchiolitis."

Emphysema is never clearly defined (a wise move) but overdistension and alveolar wall changes secondary to bronchiolitis characterize the condition with which the authors are primarily concerned. In passing, they refer to "patients exhibiting all the clinical and functional evidence of emphysema during life" but showing "remarkably little abnormality at autopsy," and conclude that "the clinician's definition must largely be based upon the disturbance in pulmonary function which is fundamental to the emphysematous process, rather than on anatomical changes." One wishes the authors had simply stated that there is more than one mechanism of diffuse airway obstruction and more than one pathogenesis of emphysema—in fact, more than one disease parading under that name.

Two chapters are devoted to emphysema and the function of the lungs. In an interesting study of function tests performed on 168 patients with chronic bronchitis, pneumoconiosis, bronchiectasis, or asthma, it was found that "although the tests could be interrelated there was no relationship between the results and the clinical syndromes which could be used in a diagnostic sense." In other words, pulmonary function tests define the severity of the physiologic derangement and not its cause.

Following excellent chapters on cor pulmonale and on the treatment of the entire group of conditions with which the book deals, comes a fascinating final chapter on epidemiology and pathogenesis. The results of symptomatic surveys are analyzed from the point of view of age, past illnesses, family history, smoking, occupation, and environment. Here the authors more than succeed in their modest hopes "that the familiar story (of obstructive disease) may be seen in its wider perspective."

RICHARD L. RILEY
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CHIRURGIE D'EXERESE DANS LA TUBERCULOSE PULMONAIRE. By D. Honoré. Pp. 216, Masson et Cie, Paris, 1958. Price, 3,300 fr.

This 216-page publication describes technique, indications, and results in 585 patients with surgically-treated pulmonary tuberculosis at the University of Liège from January, 1949, through December, 1954. The 74 tables and charts illustrate the author's detailed study of the significance of the incidence and resistance of tubercle bacilli in the sputum of pre- and postoperative patients.

This excellent monograph is a detailed account of a well-planned aggressive program for the surgical treatment of pulmonary tuberculosis. The excellent results and low mortality testify to the soundness of the operative criteria and the careful selection of patients. The correlation of bacterial study with end results, thoughtful evaluation of avoidable deaths, and careful analysis to which the material is subjected throughout the text make this a significant work.

According to the author, this monograph fulfills a need because there are no previously published studies correlating the incidence and antimicrobial resistance of tubercle bacilli in the sputum with the incidence and prognosis of bronchopleural fistula; the relation of postoperative sputum status to parenchymatous recurrence and to the end results of segmental resection.

One of the most important contributions of this

work is the demonstrated relationship of quantitative positivity of sputum and antimicrobial resistance of its tubercle bacilli to postoperative cures, relapses, complications, and deaths. These studies will lead to a more careful selection of surgical patients.

After an introductory history of the treatment of pulmonary tuberculosis, the author outlines his surgical program in detail: ventral position is used to avoid spread, thoracic cage expansion and diaphragmatic excursion are improved, and mediastinal flutter and cardiac arrhythmias are minimized. The justification of intratracheal anesthesia is overdetailed and labored.

In the section on operative technique, the author describes certain technical details to facilitate operative technique. Line drawings illustrate the hilar anatomy adequately. The author believes that extreme, sudden hypotension associated with the dissection of the costovertebral parietal pleura is secondary to interruption of sympathetic nervous system fibers, and recommends prompt treatment with vasopressor agents.

The next three chapters describe surgical indications, pre- and postoperative use of antimicrobial drugs, and the bacteriologic techniques for culture and sensitivity of the patient's sputum.

In Chapter VII, the author devotes 94 pages to his postoperative results. He discusses these in terms of early postoperative deaths, early and late bronchopleural fistula, early and late empyema, the relation of antituberculous drugs to the amount of resected lung, parenchymal recurrence, with special relationship to the bacteriologic studies, postoperative bacteriologic results, and postoperative results in relation to surgical indications. Ten pages of discussion then follow in Chapter VIII. His conclusions are clearly and simply stated in Chapter IX.

The excellence of Professor Honoré's work necessitates a summary for those interested in the surgical treatment of pulmonary tuberculosis who cannot read French or do not have access to his monograph. Patients were followed jointly by the medical and surgical services at the University of Liége. They were prepared for three to four months, or until maximal benefit was obtained with streptomycin, PAS, or isoniazid, according to their *in vitro* sensitivity response. They were classified as "resistant," "partially resistant (positive, scanty growth, negative)," and "sensitive (positive, scanty growth, negative)."

The total cure rate is 91 per cent for the entire series at the end of the minimal two years. This varies from 73 per cent among the totally resistant patients to 97.6 per cent of 84 "open-negative" patients.

Two surgical indications, not common in this country, were followed by excellent results. Thirteen patients aged seven to nineteen years with extending primary infection had uneventful resections despite technical difficulties in dissecting the hilum. Four of five "desperate-risk" patients with acute, spreading caseous pneumonia refractory to antimicrobial treatment were salvaged without mortality.

Mowlem and associates, in a numerically comparable study, did not operate on patients with positive gastric cultures unless the enlargement of their antituberculous refractory lesions required prompt removal of a focus of spread. In contrast to the Mowlem series, bacterial positivity per se was no deterrent to resection in the Liége series; perhaps the Liége series is more aggressively handled than that from the Anoka State Hospital because the former may be composed of generally better clinical material.

There were 20 deaths of 585 cases (3.4 per cent). The author's opinion of the seven avoidable deaths is stated. Two patients died from postoperative respiratory insufficiency after being operated on for acute tension penumothorax. These died of contralateral peripheral scattered atelectasis. One hemorrhage secondary to a broken suture was considered avoidable by the use of braided sutures. Three deaths from vascular collapse after decortication were attributed to nonuse of vasopressor drugs when the sympathetic nervous system was traumatized. There was one death after transfusion error. The author states that electrocardiographic and pulmonary function tests are at times unable to predict the individual outcome of certain specific cases and that patients more than fifty years old fall into the "aged category," something not necessarily agreed on in this country regarding tuberculous patients.

This careful and voluminous study leads to several conclusions. The frequency and prognosis of bronchopleural fistula and empyema are linked directly to the number and sensitivity of bacteria in the patient's preoperative sputum. Among the patients with sputum negative for tubercle bacilli at operation, there was a 1 per cent relapse rate in two years, if the unresected parenchyma was free of minor lesions. If the remaining parenchyma was not free, the relapse rate rose to 5 per cent. Segmental or atypical resections were safe in patients with negative sputum. In patients whose sputum was of equal bacterial positivity, the results were similar regardless of the indication for operation. Preoperative bacteriologic studies were valuable in the choice of therapy and in indicating the time of maximal operative safety with greatest chance of definitive cure. Postoperative bacterial study was just as necessary because sputum consistently positive for tubercle bacilli implied a greater risk

of late bronchopleural complications or parenchymal recurrence. A consistently positive postoperative sputum indicated the necessity of continued watchfulness and necessitated either resumption of antituberculous drugs, increase of administered dosage, or even a change of medication.

KEITH MERRILL, JR.
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ÜBER DEN EINFLUSS VON PHYSIKALISCHEN UND CHEMISCHEN FAKTOREN AUF DI CYTOLOGI DER TUBERKELBAZILLEN UND ANDERER MYCOBAKTERIEN. By Werner Roth. Pp. 77, Verlag Helbing & Luchtenhahn, Basel, 1958. Price, SFr. 11.50.

In the past three decades the study of morphology and cytology of bacteria received a considerable boost by the development of the electron microscope and the phase-contrast microscope. Doctor Roth's book presents a report of the current knowledge of the cytology and morphology of Mycobacteria since the advent of these advances.

The 77-page book is divided into two major sections. Following an appropriate introduction there is a chapter on the cytology of bacteria in general and another limited to a more detailed description of mycobacteria. The second is a report of the experimental studies which principally involved investigations of the influence of various physical and chemical variables on *M. smegmatis*. These were observed under the phase-contrast microscope. Detailed information is presented concerning the effect of variation in osmotic pressure, pH of the medium, and incubation temperature. The influence of disinfectants and various chemotherapeutic agents are also reported. A brief discussion and summary are presented in the last chapter.

The book is illustrated with 37 good photomicrographs and contains a bibliography of approximately 200 excellent references.

HERMAN R. HUEMPFNER
New York, New York

BOOKS RECEIVED

LONG-TERM ILLNESS. MANAGEMENT OF THE CHRONICALLY ILL PATIENT. Edited by Michael G. Wohl, M.D. Pp. 748, W. B. Saunders Company, Philadelphia, 1958.

AMINO ACIDS AND PEPTIDES WITH ANTIMETABOLIC ACTIVITY. CIBA FOUNDATION SYMPOSIUM. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 286, Little, Brown & Company, Boston, 1958. Price, $8.75.

GLASGOW'S X-RAY CAMPAIGN AGAINST TUBERCULOSIS. 11th March–12th April, 1957. Pp. 112, Glasgow Corporation Printing and Stationery Department, Glasgow, Scotland, 1958.

ANTIBIOTICS MONOGRAPHS NO. 11: MODERN CHEMOTHERAPY OF TUBERCULOSIS. Roger S. Mitchell and J. Carroll Bell. Pp. 109, Medical Encyclopedia, Inc., New York, 1958.

ERGEBNISSE DER GESAMTEN TUBERKULOSE- UND LUNGENFORSCHUNG. Edited by St. Engel, L. Heilmeyer, J. Hein, and E. Uehlinger. Pp. 735, Georg Thieme Verlag, Stuttgart, 1958. Price, $34.30.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved.

1959—Chicago, Illinois—May 24–29

1960—Los Angeles, California—May 15–20

THIRD INTERNATIONAL CONGRESS OF SCHOOL AND UNIVERSITY HEALTH

The Third International Congress of School and University Health will take place at Unesco House, Place Fontenoy, Paris 7, France, on July 6, 7, and 8, 1959. This Congress is under the patronage of the French Ministry of Education and Ministry of Health, as well as that of the International Association of Universities and the International Association for Educational and Vocational Information. It is expected that the World Health Organization and Unesco will also offer their patronage.

Requests for information may be addressed to the Organizing Committee of the Third International Congress of School and University Health, 13 rue du Four, Paris 6, France.

SECOND SYMPOSIUM ON GNOTOBIOTIC TECHNOLOGY: THE REARING AND USE OF GERM-FREE ANIMALS

A national symposium on the technology of germ-free research will be held on Wednesday and Thursday, April 8 and 9, 1959, at Lobund Institute, University of Notre Dame, South Bend, Indiana. The sponsoring institutions, in addition to Lobund Institute, are the Office of Naval Research, the Institute of Laboratory Animal Resources (National Academy of Sciences—National Research Council), and the National Institutes of Health of the U. S. Public Health Service.

Further information is available from Professor Philip C. Trexler, Lobund Institute, University of Notre Dame, South Bend, Indiana, and Donald L. Snow, Chief, Sanitary Engineering Branch, Division of Research Services, National Institutes of Health, Bethesda, Maryland.

JAMES ALEXANDER MILLER FELLOWSHIP—1959–1960

The James Alexander Miller Fellowship, established by the New York Tuberculosis and Health Association in 1949, has been awarded to Alfred J. Crowle of the Colorado Foundation for Research in Tuberculosis, Denver, for a one-year period starting on July 1, 1959. Dr. Crowle's work is related to the search for a vaccine which will offer complete protection against tuberculosis.